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AUGUST/AOÛT 9-14, 1987 EDMONTON, ALBERTA, CANADA

ABSTRACTS

RÉSUMÉS

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VII INTERNATIONAL CONGRESS OF VIROLOGY

August 9-14, 1987
Edmonton, Canada

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RÉSUMÉS
VII CONGRÈS INTERNATIONAL DE VIROLOGIE

du 9 au 14 août 1987
Edmonton, Canada

SOUS LES AUSPICES DE

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The abstracts in this volume are assembled by the type of presentation and topics. The numbering system used for the abstracts serves to identify the Plenary presentations (P), the Symposium presentations (S), the Round Table posters (R), the Open posters (OP) and the Satellite Symposium (SS).
Example:

P1.1	Plenary 1; Paper 1
S15.4	Symposium, Topic 15; Paper 4
R7.8	Round Table, Topic 7; Poster 8
OP21.19	Open Poster, Topic 21; Poster 19
SS1.1	Satellite Symposium 1; Paper 1

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Les résumés reproduits dans le présent volume sont regroupés suivant le type de présentation et le sujet. Le système de numérotage utilisé sert à identifier les présentations en séances plénières (P), les présentations faites dans le cadre de symposiums (S), les affiches pour discussions en table ronde (R), les séances d'affiches ouvertes (OP) et le symposium satellite (SS).
Exemple:

P.1.1	Plénière 1; communication 1
S15.4	Symposium, sujet 15; communication 4
R7.8	Table ronde, sujet 7; communication 8
OP21.19	Affiche ouverte, sujet 21; communication 19
SS1.1	Symposium satellite 1; communication 1

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		Round Table Poster and Open Poster Sessions (15:00-16:30) Posters on display from 10:00 Monday to 19:00 Tuesday Boards 1-368		Boards 369-800	A&B	Round Table Poster and Open Poster Sessions (15:00-16:30) Posters on display from 10:00 Wednesday to 18:00 Friday Boards 1-260		Boards 261-516		Boards 517-800	A&B
		Round Table Discussions (16:30-18:00)		Round Table Discussions (16:30-18:00)		Round Table Discussions (16:30-18:00)		Round Table Discussions (16:30-18:00)		Round Table Discussions (16:30-18:00)	
		R1 New vaccines	12	R9 Cell transformation by RNA viruses	9	R4 Virus infections of the central nervous system	8	R16 Picorna and caliciviruses	C	R3 Arena- and Bunyaviruses	12
		R2 Cell transformation by DNA viruses	3	R12 Plant virus and viroid detection using nucleic acid probes	3	R5 Non-arthropod borne Togaviruses	4	R18 Scrapie and spongiform encephalopathies	4	R8 Respiratory virus diseases	9
		R6 Replication of plant viruses	4	R13 Phages of industrial bacteria	11	R15 Viruses as genetic vectors	3	R20 AIDS: Clinical and epidemiological aspects	10	R21 Pathogenesis of viral diseases	C
		R7 Phage genetics	11	R14 Immunoregulatory responses	10	R19 Viruses of fungi	9	R22 Papillomaviruses	9	R30 Viral diagnosis: nucleic acid probes	8
		R10 Arthropod-borne Toga- and Flaviviruses	8	R17 Hepatitis	4	R23 Viral diagnosis: immunological techniques	C	R24 New plant viruses and virus diseases	3	R31 Vector transmission of plant viruses	3
		R11 Enteric virus infections	C	R25 Paramyxoviridae	8	R29 DNA and RNA insect viruses	12	R26 Taxonomy and phylogeny of phages	11	R33 Rhabdoviruses	10
		R28 Adenoviruses	10	R37 Herpes and other sexually-transmitted virus diseases	12	R35 Viruses in tropical countries	10	R27 Interferon	12	R38 AIDS: molecular biology	4
		R34 Parvovirus infections in man and animals	9			R36 DNA plant viruses	11	R32 Antiviral chemotherapy	8		
	Mixer (17:00-20:00)			Heritage Canada Concert (20:00-22:30)				BBQ (18:00-23:00)		Closing Ceremony (16:00-16:30)	C

PROGRAMME

le samedi 8 août	le dimanche 9 août	le lundi 10 août **A**	salles	le mardi 11 août **B**	salles	le mercredi 12 août **C**	salles	le jeudi 13 août **D**	salles	le vendredi 14 août **E**	salles
Inscription (13h à 18h)	Inscription (10h à 18h)	Cérémonies d'ouverture (8h30 à 9h30)	C	Séance plénière (8h30 à 11h30)		Séance plénière (8h30 à 11h30)		Séance plénière (8h30 à 11h30)		Séance plénière (8h30 à 11h30)	
		Séances plénière (9h30 à 12h)		P2 – Structure et expression des gènes viraux	C	P3 – Virus du SIDA et autres lentivirus	C	P4 – Structure moléculaire des virus	C	P5 – Les mécanismes moleculaires de la pathogénèse des infections virales	C
		P1 – Nouveaux vaccins	C					SS1 – Carrier-Mediated Antiviral Therapy	8		
		Déjeuner (12h à 13h)		Déjeuner (11h30 à 13h)		Déjeuner (11h30 à 13h)		Déjeuner (11h30 à 13h)		Déjeuner (11h30 à 13h)	
		Symposiums (13h à 15h)		Symposiums (13h à 15h)		Symposiums (13h à 15h)		Symposiums (13h à 15h)		Symposiums (13h à 15h)	
		S1 Orthomyxovirus	12	S4 Chimiothérapie antivirale	4	S15 Immunité cellulaire antivirale	10	S21 Diagnostic des infections virales au moyen de la détection des antigènes	8	S14 Contamination par les mycoplasmes et problèmes connexes de virologie	9
		S2 Transformations cellulaires par les virus	11	S8 Immunologie moléculaire des virus	10	S16 Techniques de l'ADN recombinant et vecteurs viraux	9	S24 Rétrovirus : réplication et transmission	10	S22 Immunopathologie virale	8
		S3 Aréna- et bunyavirus	4	S9 Pox- et iridovirus	8	S17 Virus à ARN : brin positif	8	S25 Virus à ARN à double brin	12	S23 Virus à ADN des plantes et vecteurs génétiques	11
		S5 Infection virale des plantes : étapes précoces et réponse de l'hôte	8	S10 Paramyxoviridae	12	S18 Virus de l'herpès	12	S26 Nouveaux phages et écologie des phages	11	S27 Coronavirus	12
				S12 Virus cryptiques des plantes	3	S19 Viroïdes et satellites	4	S30 Rhabdovirus	4	S28 Virus à ADN à réplication nucléaire	4
		S6 Virus des animaux marins et aquatiques	3	S13 Problématique de l'organisation et de l'expression du génome des phages	9	S20 Structure des phages dans les interactions avec les cellules hôtes	11	S31 Virus à ADN et à ARN des insectes	9	S29 Épidémiologie comparative des viroses	10
		S7 Hépatite	10			S33 Séquençage : taxonomie et aspects évolutifs des virus	3			S32 Évolution et écologie	3
		S11 Infections virales chez des hôtes immunodéprimés	9								
		Séances d'affichage – Tables rondes et affiches ouvertes (15h à 16h30) Affiches en étalage du lundi à 10h jusqu'au mardi à 19h Tableaux 1 à 368		Tableaux 369 à 800	A&B	Séances d'affichage – Tables rondes et affiches ouvertes (15h à 16h30) Affiches en étalage du mercredi à 10h jusqu'au vendredi à 19h Tableaux 1 à 260		Tableaux 261 à 516		Tableaux 517 à 800	A&B
		Discussions en table ronde (16h30 à 18h)		Discussions en table ronde (16h30 à 18h)		Discussions en table ronde (16h30 à 18h)		Discussions en table ronde (16h30 à 18h)		Discussions en table ronde (16h30 à 18h)	
		R1 Nouveaux vaccins	12	R9 Transformations cellulaires par des virus à ARN	9	R4 Infections virales du système nerveux central	8	R16 Picorna- et calicivirus	C	R3 Aréna- et bunyavirus	12
		R2 Transformations cellulaires par les virus à ADN	3	R12 Virus des plantes et détection de la particule virale par l'emploi de sondes d'acides nucléiques	3	R5 Togavirus non transmis par les arthropodes	4	R18 Scrapie et encéphalopathies spongiformes	4	R8 Viroses respiratoires	9
		R6 Réplication des virus des plantes	4			R15 Virus agissant comme vecteurs génétiques	3	R20 SIDA : aspects cliniques et épidémiologiques	10	R21 Pathogénèse des viroses	C
		R7 Génétique des phages	11	R13 Phages des bactéries d'usage industriel	11	R19 Virus des champignons	9	R22 Papillomavirus	9	R30 Diagnostic des viroses à l'aide de sondes d'acides nucléiques	8
		R10 Toga- et flavivirus transmis par les arthropodes	8	R14 Régulation de la réponse immunitaire	10	R23 Diagnostic des viroses : techniques immunologiques	C	R24 Nouveaux virus des plantes et viroses	3	R31 Vecteurs de transmission des virus des plantes	3
		R11 Infections entériques d'origine virale	C	R17 Hépatite	4	R29 Virus à ADN et à ARN des insectes	12	R26 Taxonomie et phylogénie des phages	11	R33 Rhabdovirus	10
		R28 Adénovirus	10	R25 Paramyxoviridae	8	R35 Virus dans les pays tropicaux	10	R27 Interféron	12	R38 SIDA : biologie moléculaire	4
		R34 Infections au parvovirus chez l'homme et l'animal	9	R37 Herpès et autres viroses transmises sexuellement	12	R36 Virus à ADN des plantes	11	R32 Chimiothérapie antivirale	8		
	Réception informelle (17h à 20h)			Concert canadien (20h à 22h30)				Barbecue (18h à 23h)		Cérémonies de clôture (18h à 18h30)	C

ABSTRACTS

RÉSUMÉS

PLENARY SESSIONS

SÉANCES PLÉNIÈRES

P1.1

VACCINATION, ACHIEVEMENTS AND PROSPECTS.
G.L. Ada, Department of Microbiology, John Curtin School of Medical Research, Australian National University, Canberra, Australia.

Vaccines against at least 50 different infectious diseases are in use in the world today. They are a highly cost-effective public and veterinary health measure. Under optimum conditions, they have caused the eradication of a disease, eg, smallpox. There are several other diseases where eradication may be possible. In other circumstances, they are less efficient, eg influenza because of unpredictable antigenic variation. There is a great variety of diseases, of viral, bacterial or parasitic aetiology, where for one or other reason, vaccines cannot be made by conventional methods, such as attenuation of the organism. These range from AIDS, caused by human immunodeficiency virus to malaria, caused by Plasmodia. There are four new approaches using molecular biological techniques which are being extensively explored. These are synthetic peptides, individual proteins, anti-idiotypes and live vectors (viruses or bacteria). Discussion will centre on the perceived advantages and drawbacks of each approach, with relevant examples. No vaccines based on these products is as yet in wide use but several are in the early stages of testing.

P1.2

VACCINIA VIRUS EXPRESSION VECTORS. B. Moss. Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892, USA.

Vaccinia virus has several advantages for use as an expression vector including a large capacity for DNA, retention of infectivity, wide host range, and extensive experience as a live smallpox vaccine. Recent advances have been made in several areas including enhancement of expression, selection of recombinant viruses, and attenuation of virus pathogenicity. The most radical innovation has been the insertion of the bacteriophage T7 RNA polymerase gene into the vaccinia virus genome thereby permitting the use of bacteriophage transcriptional regulatory signals for selective expression of foreign genes. Recombinant vaccinia viruses are now widely used to determine the targets of both humoral and cell mediated immunity. Experimental animals, inoculated with live recombinant vaccinia viruses, have been protected against challenge with the corresponding virus pathogens. Genetic engineering has also been used to attenuate the virulence of vaccinia virus in order to increase the safety of recombinants for potential human or veterinary purposes. Recent studies with recombinant vaccinia viruses that express human immunodeficiency virus genes will be described.

P1.3

THE MOLECULAR BASIS OF IMMUNITY TO INFLUENZA.
J.J. Skehel. Division of Virology, National Institute for Medical Research, Mill Hill, London NW7 1AA.

Analyses of the structure of Hong Kong influenza virus haemagglutinins have indicated regions of the molecules which bind neutralising antibodies, interact with cell membrane receptors, and which are recognised by immune T cells.

Descriptions will be given of these different sites and their inter-relationships discussed, with particular reference to influenza virus variation and vaccination.

P1.4

CATALYTIC ANTIBODIES. A.Tramontano, K.D. Janda, R.A. Lerner. Department of Molecular Biology, The Research Institute of Scripps Clinic, La Jolla, California

Monoclonal antibodies elicited to haptens that are analogs of the transition state for hydrolysis of carboxylic esters behaved as enzymic catalysts with the appropriate substrates. These substrates are distinguished by the structural congruence of both hydrolysis products with haptenic fragments. The haptens were potent inhibitors of this esterolytic activity, in agreement with their classification as transition state analogs. Mechanisms are proposed to account for the different chemical behavior of these antibodies with two types of ester substrates. The generation of an artificial enzyme through transistion state stabilization by antibodies was thus demonstrated. These studies indicate a potentially general approach to catalyst design.

P1.5

THE SCIENTIFIC BASIS OF THE STANDARDIZATION AND CONTROL OF VIRAL VACCINES. G. C. Schild, National Institutes of Biological Standardization and Control, London, UK

ABSTRACT NOT AVAILABLE

P2.1

HUMAN PAPILLOMA VIRUSES. H. zur Hausen, German Cancer Center, Heidelberg, FRG

ABSTRACT NOT AVAILABLE

P2.2

TRANSFORMATION BY EPSTEIN-BARR VIRUS. B. Sugden, V.R. Baichwal, and N. Warren. McArdle Laboratory, University of Wisconsin, Madison, Wisconsin 53706, U.S.A.

We are studying three genetic elements of Epstein-Barr Virus (EBV) that are likely to be required for its ability to immortalize human B-lymphocytes efficiently. Two of these, oriP and the gene encoding the EBNA-1 protein, together constitute an EBV plasmid replicon. The third, a membrane-associated protein, encoded by the BNLF-1 ORF, confers anchorage-independent growth on two established rodent cell lines. In addition to supporting the cis-acting oriP as an origin of plasmid replication, EBNA-1 trans-activates a transcriptional enhancer that is an essential component of oriP. This enhancer stimulates transcription from a promoter 3300bp downstream on the viral genome. It appears that some RNAs initiating from this promoter encode EBNA-1 and, therefore, EBNA-1 may regulate its own synthesis.

Wang et al (Cell 43: 831-840, 1985) demonstrated that the expression of the BNLF-1 ORF of EBV in Rat-1 cells induced their anchorage-independent growth. We have extended these results to BALB 3T3 cells and have found that this viral protein is turned over in EBV-immortalized lymphoblasts with a half-life of 5 hrs. and in anchorage-independent BALB 3T3 cells with a half-life of 2.5 hrs. The protein is phosphorylated at serines in lymphoblasts but it is not detectably phosphorylated in anchorage-independent BALB 3T3 cells. A deletion analysis of the BNLF-1 ORF indicates that the membrane-spanning domain of BNLF-1 mediates the induction of the phenotype of anchorage-independent growth in BALB 3T3 cells.

P2.3

ORGANIZATION AND EXPRESSION OF CORONAVIRUS GENOMES

S. Siddell
Institute of Virology, University of Würzburg, 8700 Würzburg, F.R.G.

The Coronaviridae are a monogeneric family of enveloped, positive-stranded RNA viruses which infect vertebrates and cause diseases of economic importance. This presentation will review the organization and expression of coronavirus genomes.

Firstly, the synthesis of coronavirus RNAs, in particular, the synthesis of subgenomic mRNAs, will be described. Characteristically, coronavirus gene expression is mediated by a 3' coterminal set of mRNAs whose synthesis involves an unusual mechanism of discontinuous transcription. Secondly, the synthesis of coronavirus proteins will be considered. Data to support a model in which translation occurs only from the 5' unique region of each mRNA, a non-overlapping translation strategy, will be presented. Finally, the genomic organization of avian, murine, porcine, feline and bovine coronaviruses will be compared.

P2.4

REPLICATION AND EXPRESSION OF BROMOVIRUS GENES. Paul Ahlquist, Biophysics Laboratory and Plant Pathology Department, University of Wisconsin, Madison, WI 53706, USA.

Brome mosaic virus (BMV) has a genome divided among three (+)ssRNAs and shares nonstructural protein homologies, replication and gene expression strategies with many diverse plant viruses and the animal alphaviruses. Divided genomes, efficient RNA transfection and other plant virus properties provide substantial advantages in many studies of gene function and expression with this virus class. The availability of infectious in vitro transcripts from cloned cDNA further facilitates studies with BMV.

Functions of the two BMV genes required for RNA replication have been investigated by targeted mutagenesis, producing distinctive ts RNA replication phenotypes in both genes. Cis-acting signals for BMV RNA replication, involving both terminal and internal noncoding sequences, have also been defined. The internal promoter for RNA-directed synthesis of BMV coat protein mRNA has been studied in vivo and separated into subdomains governing initiation site and activity level. Multiple copies of this promoter have been engineered into BMV to produce new, differentially regulated mRNAs during infection. Foreign genes under control of the promoter can be replicated and efficiently expressed in vivo in BMV-based RNA gene vectors. Recently, we have also made infectious in vitro transcripts from cDNA clones of cowpea chlorotic mottle virus (CCMV), a related virus differing in host range and other traits. Properties of hybrid viruses involving gene exchanges among BMV, CCMV and tobacco mosaic virus will be discussed.

P2.5

REGULATION OF ADENOVIRUS GENE EXPRESSION. T. Shenk, Department of Molecular Biology, Princeton University, Princeton, NJ 98544, USA

Mutational analysis of the adenovirus genome has provided insight to mechanisms regulating viral and cellular gene expression at a variety of levels.

The adenovirus E1A gene products regulate expression at the level of transcription. The E1A gene is autoregulated by its products. A cellular factor has been identified that binds to a repeated sequence within the E1A enhancer domain. It is present in both infected and uninfected cells. However, the complex of infected-cell factor bound to DNA displays an altered electrophoretic mobility as compared to the uninfected-cell factor/DNA complex. The E1A gene product appears to alter the factor either directly or indirectly.

The 55kd polypeptide encoded by the adenovirus E1B gene functions post-transcriptionally to regulate cytoplasmic accumulation of both viral and cellular mRNAs late after infection. It appears to facilitate movement of viral mRNAs from nucleus to cytoplasm while inhibiting the same process for cellular mRNAs.

The adenovirus-coded VAI RNA is required for efficient translation late during the viral growth cycle. It functions by preventing activation of the interferon-induced eIF-2α kinase. VAI RNA is responsible, at least in part, for the failure of α- or β-interferons to inhibit growth of adenoviruses.

P3.1

A COMPARATIVE ANALYSIS OF THE GENOMES OF THE HUMAN AND SIMIAN IMMUNODEFICIENCY VIRUSES. *M. Guyader*[1], M. Emerman[1], L. Chakrabarti[2], P. Sonigo[2], L. Montagnier[1], and M. Alizon[1]. 1 : Unité d'Oncologie Virale, 2 : UREG, Institut Pasteur, Paris, France

The human acquired immune deficiency syndrome (AIDS) is caused by two different retroviruses designated "human immune deficiency virus type 1 and 2" (HIV-1 and 2). This latter virus appears to be closely related to the simian immune deficiency viruses (SIV) a group of cytopathic retroviruses whose prototype, SIV_{mac}, was identified in captive macaques monkeys with a naturally occurring and experimentally transmittible AIDS-like disease. The HIVs and SIV share their essential biological properties : cytopathic effects in vitro, and tropism for the CD4 (T4) cells ; they are structurally related, and their proteins display a certain extent of antigenical cross-reactivity. The comparative analysis of their genomes therefore represents a potential help to delineate the conserved domains of the viral proteins and regulating elements, and towards understanding their structure-function relationships.

The genomes of HIV-2 (isolate ROD) and SIV_{mac} (Mm 142-83) have been cloned and sequenced, and compared to previously characterized HIV-1 isolates. HIV-2 and SIV appear to be about 80 % homologous in terms of nucleotide sequence, but both only share some 45 % of homology with HIV-1. This distant relationship suggests the existence of HIV relatives in man long before the current AIDS epidemics.

P3.2

HIV GENES AND THEIR FUNCTIONS. A.G. Fisher, F. Wong-Staal, & colleagues, LTCB, NCI, NIH, Bethesda, MD 20205, USA In vitro mutagenesis of biologically active molecular clones of HIV are powerfuls tools in dissecting the functions of different elements of AIDS virus. To date, eight genes have been defined within the genome of this virus. Three encode structural products of the retrovirus (gag, pol, env). The remainder code for regulatory proteins (tat, trs/art) or proteins of an undefined function (sor, R, 3'orf). Analysis of tat-III, trs/art deletion mutants have shown that these genes are required for replication and are dual-functional (influencing viral transcription and translation). Similar approaches have recently shown that the sor gene, although not required for virion formation per se, is a requisite for infectivity; sor mutant virus is at least one hundred times less infectious than intact strains (when transmitted in cell free manner). Several different hypothesis to account for the enhancement of virus infectivity of sor will be discussed. Analysis of the R and 3'orf genes of HIV indicate that the genes are dispensible for the cytopathic and replicative properties of HIV in vitro. Mutations within the env gene of HIV and analysis of hybrid molecular clones (containing a substituted env gene) show that relatively 'minor' disturbances of the env gene radically affect the binding, entry, replication and cytotoxic poperties of progeny virus as well as their sensitivity to neutralization by antisera. The biological aspects of these envelope mutants will be discussed.

P3.3

RECEPTOR MOLECULES FOR HIV. S. McDougal. Centers for Disease Control, Atlanta, GA U.S.A.

Human immunodeficiency virus (HIV) is cytopathic for CD4 T cells and binds to these cells through a complex of the 110K (or 120K) viral envelope glycoprotein gp110 (or gp120) and the CD4 molecule. We briefly review evidence that the CD4 molecule functions as a receptor for HIV. Various physical, chemical, and enzymic treatments of HIV indicate that there are conformational and structural constraints on the virus with respect to its capacity to bind to CD4. Results from binding inhibition studies using polyclonal and monoclonal antibodies, synthetic peptides, and anti- (anti-CD4) idiotypic reagents and their implications for experimental approaches to defining the binding sites on gp110 and CD4 are presented.

P3.4

AIDS IN THE TROPICS. P. Piot. Institute of Tropical Medicine. Antwerp, Belgium.

AIDS and HIV infection have become important public health problems in the tropics, particularly in sub-Saharan Africa and the Caribbean, where annual incidence rates between 170 and 880 cases per million inhabitants have been reported. Seroprevalence rates in the general population in Africa range from 0.5 % to 18 % among blood donors, and are as high as 27 % to 88 % in high risk groups such as prostitutes. The major modes of transmission include bidirectional heterosexual contact, perinatal mother-to-neonate transmission, blood transfusion, and probably contaminated injections. The epidemiological picture has become further complicated by the emergence of HIV-related retroviruses in West Africa. AIDS will probably have an impact on health care programmes and economic development in many developing countries, and its control should be a public health priority.

P4.1

STRUCTURE OF ISOSAHEDRAL VIRUS PARTICLES - AN OVERVIEW. Stephen C. Harrison, Howard Hughes Medical Institute and Dept. of Biochemistry & Molecular Biology, Harvard University, Cambridge, MA 02138.....

The way a virus particle is constructed embodies a strategy for assembly as well as for entry and uncoating. Structures of a number of isosahedral RNA plant viruses and small RNA animal viruses have now been determined by high-resolution X-ray crystallography. They show functionally important similarities. All are based on a common subunit design: essential features are a principal domain that forms the viral shell and an N-terminal extension that forms an internal scaffold; variable features are surface loops between β strands of the principal domain and C terminal extensions. Accurate assembly is determined by the N-terminal arms. The pattern of interactions is different in the T=3 plant viruses and in the picornviruses, corresponding to different assembly pathways. The T=3 plant viruses have a defined expanded state of the virion, which may be an uncoating intermediate. The compact-expanded transition is regulated by Ca^{++} binding at certain subunit interfaces. A subset of the N-terminal arms can loop out through gaps in the shell of expanded virions; loss of VP4 when picornviruses bind to cells may reflect a similar structural transition. Properties exhibited by the small, positive-strand RNA viruses - an internal scaffold, a concerted structural transition, Ca^{++} regulation - may also characterize more complex icosahedral particles, such as polyomaviruses and reoviruses.

P4.2

PICORNAVIRUSES. Michael G. Rossmann. Department of Biological Sciences, Purdue University, West Lafayette, Indiana 47907, USA.

The structure of the protein shell of a number of simple *icosahedral RNA plant and animal* viruses are now known at atomic resolution. These structures have provided a wealth of information on viral assembly, viral disassembly, the antigenic surface on viruses available to neutralizing antibodies, the host cell receptor attachment site, processing of polyproteins during maturation and the manner in which antiviral agents can interfere with the function of a viral capsid.

P4.3

THE STRUCTURE OF A COMPLEX BETWEEN THE HAEMOGGLUTININS OF INFLUENZA VIRUS AND A CELLULAR RECEPTOR, D.C. Wiley, Howard Hughes Medical Institute and Dept. of Biochemistry and Molecular Biology, Harvard University, Cambridge, MA, USA

The three dimensional structures of complexes between influenza virus haemagglutinins and trisacchaside analogs of their cellular receptors have been determined by X-ray crystallography. Analysis of the structure of the HA-receptor complex indicates (Weis, et al): 1) Sialic acid is the dominant component of the influenza virus receptor; 2) Sialic acid binds to HA residues and atoms conserved during antigenic drift; 3) The major mechanism of neutralization of influenza virus by antibodies from blocking receptor binding.

The description of the HA-cellular receptor interaction suggests approaches to the design of antiviral drugs which would inhibit virus attachment to cellular receptors. NMR binding constant measurements and a synthetic chemistry strategy will be discussed aimed at better understanding the binding.

Site directed mutations have been designed based on the HA studies of the influenza HA which have resulted in molecules with altered and abolished membrane-fusion activity. These mutations further describe the HA conformational change required for membrane-fusion and suggest a strategy for inhibiting fusion (Pfeifer, et al).

P4.4

THE THREE-DIMENSIONAL STRUCTURE OF A COMPLEX OF INFLUENZA VIRUS NEURAMINIDASE AND AN ANTIBODY. P.M. Colman, W.G. Laver (1), J.N. Varghese, A.T. Baker, P.A. Tulloch, G.M. Air (2) and R.G. Webster (3). CSIRO, Division of Protein Chemistry, 343 Royal Parade, Parkville, 3052, Australia. (1) John Curtin School of Medical Research, Australian National University, Canberra. (2) Department of Microbiology, University of Alabama, Birmingham, Alabama. (3) St. Jude Childrens Research Hospital, Memphis, Tennessee.

X-ray diffraction analysis of crystals of a monoclonal Fab fragment NC41 bound to a viral antigen, influenza virus neuraminidase, shows an epitope involving 4 surface loops of the antigen. The structure of the Fab in the complex reveals an unusual pairing pattern between the domains of light and heavy chains in the variable module of the antibody. This we interpret as implying that association with antigen can induce changes in the quaternary structure of the Fab, through a sliding of domains at the Vl-VH interface. In addition, Fab binding has altered the conformation of one of the surface loops of the antigen, shifting the position of an active-site arginine (371) and providing a possible, but unproven, explanation for the low neuraminidase activity of this complex in which the active centre remains accessible to small substrates.

P4.5

THE STRUCTURE OF THE BIPARTITE RNA INSECT VIRUS BBV. J. E. Johnson[1], H. V. Hosur[1], T. Schmidt[1], R. C. Tucker[1], T. M. Gallagher[2], B. M. Selling[2] and R. R. Rueckert[2]. [1]Department of Biological Sciences, Purdue University, West Lafayette, USA. [2]Biophysics Laboratory, University of Wisconsin, Madison, USA.

We report the first atomic resolution study of an insect virus, black beetle virus, which has a bipartite RNA genome encapsulated in a single particle. The capsid contains 180 protomers arranged on a T=3 surface lattice. The quaternary organization of the protomers is nearly identical to that observed in the T=3 plant virus structures. The protomers consist of three visible domains, a β-barrel as seen in other animal and plant virus subunits, an outer domain composed predominantly of β-sheet and formed by three large insertions between strands of the barrel and a domain composed of two distorted helices lying inside the shell. The outer domains of quasi threefold related protomers form pyramidyl surface protrusions. Both termini, 64 residues at the amino end and 62 at the carboxy end, lie inside the shell but are crystallographically disordered and therefore invisible.

P4.6

ADENOVIRUS ARCHITECTURE. Roger M. Burnett. Department of Biochemistry & Molecular Biophysics, Columbia University, New York, New York, U.S.A.

The adenovirus virion is over an order of magnitude larger than the RNA viruses whose structures have been determined by X-ray crystallography. The architecture is being explored by a combination of X-ray crystallography, electron microscopy and biochemistry. The crystal structure of hexon, the major coat protein, has been determined. Each 967 residue subunit of the hexon trimer contains two beta-barrels with identical morphology to those in the RNA viruses. A common ancestral gene for all viral coat proteins is indicated. Electron micrographs of various capsid fragments show the organization of the outer shell. Hexons form a small p3 crystal on each facet in which at least 2/3 of their inter-hexon bonds are identical. A biochemical analysis of the virion proteins has provided the absolute stoichiometry for the 2700 structural polypeptides, defined the structure of the penton complex at the vertex and indicated how its puzzling symmetry mismatch is overcome. The capsid dissociation pattern is explained by the binding of polypeptide IX as a cement. Its sites are revealed by subtracting the X-ray hexon model from electron micrographs of facet fragments. A general principle emerges that large, weakly-interacting, building blocks (e.g. hexon) permit accurate assembly. Stability is conferred by later addition of different structural components (e.g. polypeptide IX). (Supported by NIH AI 17270 and NSF PCM 84-18111).

P5.1

THE ATTENUATION OF POLIOVIRUS NEUROVIRULENCE J.W. Almond, G.D. Westrop, K. Wareham, G.C. Schild* and P.D. Minor*, Department of Microbiology, University of Reading, U.K. and *NIBSC, Holly Hill, Hampstead, London.

Although the live-attenuated oral poliovirus vaccines are very effective, it is clear that in certain rare instances they can revert to neurovirulence and cause poliomyelitis in recipients. Our work has concentrated mainly on the poliovirus type 3 vaccine since this is the one most frequently implicated as a cause of vaccine associated disease. Nucleotide sequence studies on the vaccine strain, P3/Leon12alb, its neurovirulent parent P3/Leon/37, and an isolate from a vaccine associated case of poliomyelitis, P3/119, has revealed all of the mutations which must account for the attenuated phenotype of the vaccine and the reversion to neurovirulence of P3/119. Partial sequence analysis of a number of other vaccine revertants and on virus recovered from healthy vaccinated children, has provided further information on the mutations involved in attenuation and reversion. Final analysis of the role of each mutation is provided by the construction in vitro of defined recombinants using infectious cDNAs. Our results suggest that attenuation of the Sabin type 3 poliovirus vaccine is effected by just two base changes. The likely mechanisms of action of these mutations will be discussed.

P5.2

INTERFERON- INDUCED CELLULAR PROTEINS AS SPECIFIC COMPONENTS OF THE ANTIVIRAL STATE. O. Haller, Institute for Immunology and Virology, University of Zürich, Zürich, Switzerland.
Interferons (IFNs) induce in cells the synthesis of several proteins which are thought to be mediators of the multiple effects of IFNs. Transcription of host cell genes is activated within minutes following IFN binding to specific cell surface receptors. However, the biological role of many of the induced gene products is still unknown. A classical approach to clarify the different functions of IFN- induced proteins is to study host cell mutants lacking one or the other of these molecules. Most inbred mouse strains carry deletions at one IFN-activated gene called Mx. The wild type allele is present in two inbred strains and in some wild mice. It codes for an IFN- induced 72 kD nuclear protein. The cloned murine Mx cDNA, when placed under the control of a constitutive promoter and introduced into murine Mx negative cells, leads to the accumulation of Mx protein in the nuclei and to protection against influenza virus (but not vesicular stomatitis virus) even in the absence of IFN treatment. Thus, Mx protein represents a specific component of the antiviral state being both necessary and sufficient for protection against influenza viruses.

P5.3

MOLECULAR PATHOGENESIS OF EPSTEIN BARR VIRUS (EBV) INFECTION. D. Wang, J. Sample, F. Wang, D. Braun, D. Liebowitz, J. Tanner, L. Petti and E. Kieff. University of Chicago, Chicago, IL, USA.

EBV, a herpesvirus, is the causative agent of infectious mononucleosis and a cause of B cell proliferative diseases in immunecompromised patients. In vivo or in vitro infection of B lymphocytes with EBV results in persistence of the virus genome as episomes or as integrated DNA, expression of a highly restricted class of virus genes, immunoglobulin secretion and perpetual cell proliferation. Some of the early events in B cells can be attributed to a virion outer envelope glycoprotein, gp350/220, which is a highly specific ligand for CR2. Binding to CR2 results in capping, cocapping of surface immunoglobulin, endocytosis of virus, and cell clumping and enlargement. Five EBV genes are regularly expressed in latent infection. EBV nuclear protein 1 (EBNA1) binds to an EBV DNA sequence which is necessary for maintaining DNA as episomes. EBNA2 causes lymphocytes to grow in tight clumps and is a direct or indirect transactivator of expression of the B lymphocyte surface protein CD23. EBNA3 is one of a family of three homologous tandemly placed genes all of which may be expressed in latent infection. All of the nuclear protein genes are probably transcribed from the EBNA2 promoter, and share a 1.2 kb mRNA leader sequence, which encodes a nuclear protein, EBNA-LP. The latent infection membrane protein, LMP, is transcribed leftward from a promoter near the right end of the EBV genome. LMP is a dominant transforming gene in Rat-1 or Balb/c 3T3 cells. LMP also increases DNA synthesis in immortalized lymphocytes.

P5.4

ANATOMY OF PERSISTENT VIRUS INFECTION. M.B.A. Oldstone, J.L. Whitton, M. Salvato. Viral Immunobiology Unit, Department of Immunology, Research Institute of Scripps Clinic, La Jolla, CA, USA.

For viruses to persist they need to escape the host's immune surveillance mechanism(s). To gain insights in the role of virus-lymphocytes interactions in aborting immune surveillance we mapped virally encoded epitopes recognized by cytotoxic thymus derived lymphocytes (CTL). These cells are important in terminating lymphocytic choriomeningitis virus (LCMV) infection. Their absence or dysfunction lead to persistence and their reconstitution clears viral materials and terminates persistence. LCMV is composed of two RNA segments, a large (L)=8.8 kb and small (S)=3.4 kb. Reassorting RNA segments indicates that the S RNA and the genes it encodes are implicated in generation of CTL. The S RNA encodes two genes, glycoprotein (Gp) at 5' and nucleoprotein (NP) at 3' ends. cDNA of either Gp or NP and corresponding truncations inserted into vaccinia vectors map CTL epitopes on several different MHC haplotypes. Synthesizing short peptides corresponding to the component(s) of viral protein expressed allowed fine mapping and single amino acid substitution could abort CTL activity.

P5.5

THE MOLECULAR BASIS OF INFLUENZA VIRUS PATHOGENICITY

Rudolf Rott and Hans-Dieter Klenk.
Institut für Virologie, Justus-Liebig-Universität Giessen, and Philipps-Universität Marburg, F.R.G.

Disease resulting from virus infection is a complex event, depending on the close interaction of viral and host factors. Through the application of biochemical and genetic methods it became possible to gain an insight into the molecular basis of these interactions. Thus, it has been shown that the glycoproteins of enveloped viruses play a critical role in the initiation of infection. They are responsible not only for the adsorption of virions to cellular receptors, but they are also for the entry of their genome into the cell by fusion of the viral envelope with cellular membranes. Evidence is growing that the fusogenic glycoproteins are frequently activated by cellular proteases. The structure of the proteins at the cleavage site and the availability of a suitable protease are critical for tissue tropism, spread of the virus in the infected organism and, thus, for pathogenicity.

This will be demonstrated by the example of the haemagglutinin of influenza viruses, but evidence will also be presented that similar concepts may be relevant for other viruses, too.

SYMPOSIA

SYMPOSIUMS

S1.1

THE M_2 and NB INTEGRAL MEMBRANE PROTEINS OF INFLUENZA VIRUSES. BMBCB, Northwestern University, Evanston, IL, U.S.A. Robert A. Lamb, J. David Hull, Mark A. Williams and Suzanne L. Zebedee.

The NB glycoprotein of influenza B virus is abundantly expressed at the infected cell surface. Treatment of infected cells with proteases and endoglycosidase F and immunoprecipitation with site-specific antisera, indicated that ~20 N-terminal residues containing two N-linked carbohydrate chains are exposed at the cell surface. Removal of the carbohydrate addition sites by mutagenesis, indicates that two carbohydrate chains are added to the extracellular region of NB. These sugar chains are extensively modified to form higher glycans.

Monoclonal antibodies have been produced to the N-terminal domain of the M_2 integral membran protein of influenza A virus and the antibodies used to quantitate the amount of M_2 found in purified virions. The relative abundance (and significance) of M_2 identified in virions of various strains, grown in eggs and tissue culture, will be discussed. The mechanism by which the hydrophobic domain of M_2 interacts with the membrane translocation machinery has been examined in vitro using translation systems, salt washed membranes and SRP and these data will be discussed.

S1.2

INHIBITION OF INFLUENZA A VIRUS REPLICATION BY AMANTADINE
A.J. Hay, M.H. Smith, M.C. Zambon, G. Bahadur, R. Sugrue and M. Bouzyk, Division of Virology, National Institute for Medical Research, London, U.K.

The primary target of the anti-influenza A drugs amantadine and rimantadine has been identified as the transmembrane domain of the M2 protein. The haemagglutinin (HA) has also been implicated in drug action since this virus component can influence both the degree of virus susceptibility and the stage of replication inhibited, either entry of virus into cells or virus maturation. More significantly, in the latter case the HA itself is modified by drug treatment such that molecules expressed on the surface of infected cells have a conformation more akin to that induced by low pH treatment. Since alterations in only M2 are sufficient to abolish this effect upon HA, it appears that the drug interferes with an interaction between these two proteins which occurs during maturation and transport of HA to the plasma membrane.

S1.3

FUNCTIONS OF THE INFLUENZA C VIRUS GLYCOPROTEIN
G. Herrler and H.-D. Klenk, Institut für Virologie, Philipps-Universität, 3550 Marburg, FRG.

In contrast to other myxoviruses, only one surface glycoprotein (gp) has been detected on influenza C virions which is known to mediate fusion of the viral envelope with cellular membranes at low pH. We have obtained evidence that gp represents also the receptor-destroying enzyme of influenza C virus which has been characterized as a neuraminate 9-O-acetyl-esterase: (i) purified gp is able to inactivate cellular receptors for influenza C virus; (ii) purified gp was found to have acetylesterase activity ; (iii) monoclonal antibodies directed against gp inhibit the acetylesterase activity of influenza C virus. Though purified gp was unable to agglutinate chicken red blood cells, it was shown to have hemagglutination-inhibition (HI) activity. This finding as well as the HI-activity of monoclonal anti-gp antibodies indicate that gp is also responsible for the hemagglutinating activity of influenza C virus. Unlike fusion, hemagglutination and receptor destroying activity do not depend on proteolytic cleavage of gp. The surface glycoprotein of influenza C virus is therefore unique among the myxovirus glycoproteins by combining the functions of a fusion factor, a hemagglutinin, and a receptor destroying enzyme in one molecule.

S1.4

AN IDENTIFICATION OF THE ATTENUATING GENES IN COLD-ADAPTED AND AVIAN-HUMAN INFLUENZA A REASSORTANT VIRUSES. B. R. Murphy and M. H. Snyder, NIAID, NIH, Bethesda, MD 20892.

Influenza A viruses can be attenuated for humans by the transfer of genes that specify attenuation from a donor virus to a wild type. Two donor viruses being studied are: (1) a human influenza A/Ann Arbor/6/60 (H2N2) virus that was passaged multiple times in chick kidney tissue at low temperatures that acquired three phenotypes: (a) temperature sensitivity (ts) of replication, (b) cold-adaptation (ca), and (c) attenuation (att) in animals and man; (2) an avian influenza A virus, A/Mallard/New York/78 (H2N2), that is restricted in replication in the lower respiratory tract of non-human primates. Reassortant viruses containing the HA and NA surface antigens of a human virus and the other six RNA segments from either donor are safe, immunogenic and efficacious in humans. A determination of the genetic basis for the phenotypes of the two donor viruses has been revealed for the A/AA/6/60 ca donor virus that (1) the PB2 gene is the major gene conferring the ts phenotype, but the PB1 genes also contributes; (2) the PA gene specifies the ca phenotype; (3) the PA gene specifies the att phenotype in humans with contributions by the M, and perhaps NP, and PB2 genes. For the avian influenza A donor virus both the NP and M genes contribute to att in squirrel monkeys. Thus at least two genes contribute to the att phenotype manifested by each donor virus.

S1.5

ENZYMATIC AND ANTIGENIC ACTIVITY OF THE INFLUENZA VIRUS NEURAMINIDASE. G.M. Air, M.R. Lentz, W.G. Laver*, P.M. Colman+ and R.G. Webster#, Department of Microbiology, University of Alabama at Birmingham, *Australian National University, +C.S.I.R.O. and #St. Jude's Hospital.

We have investigated some structure-function relationships in the influenza neuraminidase using monoclonal antibodies directed against epitopes on the native enzyme, the biological properties and sequence changes in variant viruses isolated naturally or selected with the monoclonal antibodies, and site-specific mutations.

The crystal structure of an antibody Fab-N9 neuraminidase complex (Colman et al.) has defined a major epitope and enabled us to interpret many of the properties of virus variants. For this epitope, single amino acid sequence changes which abolish antibody binding but which have no other detectable biological effect are all located in the interface region.

Amino acid side chains which line the enzyme active site pocket are conserved in all known neuraminidases. Site-directed mutation of 12 of these resulted in complete loss of enzyme activity, even though the changes made were rather conservative and monoclonal antibody analysis indicated that the protein was correctly folded. The pH optimum for enzyme activity was lowered when His 274 of N2 neuraminidase was altered, suggesting that catalysis involves transfer of a proton from His 274.

S1.6

TRANSCRIPTION MECHANISMS AND REGULATED PRE-mRNA SPLICING IN INFLUENZA VIRUS REPLICATION. R.M. Krug, C. St. Angelo, G. Shapiro, C. Agris, Memorial Sloan-Kettering Cancer Center, New York, NY, USA

We will discuss our recent results on the mechanism of: (i) influenza viral mRNA synthesis; (ii) viral RNA replication; and (iii) the control of viral mRNA splicing. Viral mRNA synthesis, which is initiated by capped RNA primers, is catalyzed by a complex of the three polymerase (P) proteins, PB1, PB2 and PA, associated with viral nucleocapsids. To elucidate the roles of each of the P proteins, we have synthesized large amounts of these proteins using baculovirus vectors and are attempting to reconstitute functional P protein complexes. We have established an in vitro system that catalyzes both steps in viral RNA replication: the synthesis of template RNAs, i.e., full-length copies of the virion RNAs (vRNAs) and the synthesis of vRNAs. Initiation as well as elongation of template RNAs and vRNAs was shown to occur in vitro. The nucleocapsid protein (NP) is required for the elongation of both types of RNAs. The splicing of two of the viral mRNAs, the NS1 and M1 mRNAs, is controlled such that the amount of the spliced mRNA is only 5-10% of that of its unspliced mRNA precursor. Our results indicate that splicing is controlled largely by the structure of the NS1 and M1 and mRNA precursors which contain sequences that are not conducive to efficient splicing. We are currently identifying these sequences.

S2.1

MECHANISMS OF RETROVIRAL ONCOGENESIS. Harold Varmus, Dept. of Microbiology and Immunology, University of California, San Francisco CA 94143, U.S.A.

Most oncogenic retroviruses induce tumors through the action of viral oncogenes, the transduced versions of cellular proto-oncogenes, or through the insertional activation of cellular proto-oncogenes. I will discuss our efforts to understand examples of each of these two phenomena.

(i) The v-src gene of Rous sarcoma virus encodes a membrane-associated protein-tyrosine kinase that is more active but structurally similar to the product of the proto-oncogene, c-src, and to the products of several other members of the src gene family. I will describe our attempts to use mutant and wild-type alleles of v- and c-src and the techniques of somatic cell genetics to identify host functions that affect src proteins.

(ii) The int-1 gene is a proto-oncogene first discovered as a target for insertional activation by proviruses of the mouse mammary tumor virus. The gene encodes a primary protein of 370 amino acids that is modified by glycosylation and proteolysis and appears to enter the secretory pathway. When placed in a murine leukemia virus vector, the gene induces a partially transformed phenotype in established lines of mouse mammary cells, but does not alter the behavior of rodent fibroblasts. Unlike most other proto-oncogenes, int-1 is normally expressed only in narrow developmental windows: in the neural tube between days 8 and 13 of mouse embryogenesis and in early spermatids beyond 25 days after birth.

S2.2

Oncogene Activations by Nonacute Avian Retroviruses
Hsing-Jien Kung, Department of Molecular Biology and Microbiology, Case Western Reserve University, School of Medicine, Cleveland, OH 44106

Host oncogene activation by retroviral DNA insertion is now recognized as a major pathway in oncogenesis associated with nonacute retrovirus infection. Nonacute retroviruses are naturally abundant, cancer-inducing viruses which do not carry oncogenes of their own. In the avian system, Avian Leukosis Virus (ALV) and Reticuloendotheliosis Virus (REV) are the two prototypic examples. Both ALV and REV can induce a variety of neoplasms in chickens, depending on the genetic backgrounds of the hosts. My lab has characterized the molecular basis of chicken B and T-lymphoma, and erythroblastosis induced by either ALV or REV. In every case examined, there is a strong correlation of viral DNA insertion into a specific locus and tumor induction. C-myc is found to be the target locus for ALV and REV induced B-lymphoma. C-erbB/EGF-R, on the other hand, represents the common insertion site in ALV induced erythroblastosis. These studies reveal not only the basic steps involved in oncogenic conversion of c-myc and c-erbB, but also the various means whereby retroviral DNA interacts with host gene. They also provide insight into the coordinated regulation of retroviral 5' and 3' LTR promoters.

S2.3

CELL TRANSFORMATION BY BOVINE PAPILLOMAVIRUS TYPE 1.
Douglas R. Lowy, Pamela Rawley-Nelson, Nancy L. Hubbert, Karen H. Vousden, John T. Schiller, Elliot J. Androphy. Laboratory of Cellular Oncology, National Cancer Institute, Bethesda, Maryland, USA.

The bovine papillomavirus type 1 (BPV) can induce tumorigenic transformation of mouse C127 and NIH 3T3 cells. The 8 kb genome contains two genes, E5 and E6, that can independently transform C127 cells, but only E5 transforms NIH 3T3 cells. The E5 protein product has been localized to the plasma membrane, that of E6 to nuclei and membranes. In human PV, the analogous E6 gene and its protein product are selectively expressed in cervical carcinoma cell lines.

The BPV E2 open reading frame can also significantly affect transforming activity. Rather than having intrinsic transforming activity, E2 appears to function by regulating viral transcription. A full length E2 protein binds to a specific motif present several times in the upstream regulatory region of BPV and other PVs. This motif, when present in two or more copies, serves as an E2 dependent enhancer element. Two E2 protein products have been identified in transformed cells, one a full-length (45 kd) protein, the other a truncated (31 kd) form that contains the DNA binding activity but does not enhance. The truncated version of E2 may therefore competitively inhibit enhancement by the full length E2 product.

S2.4

TRANSFORMATION BY POLYOMA VIRUS: ROLE OF THE MIDDLE T ANTIGEN:pp60^{c-src} COMPLEX. Sara A. Courtneidge and Emin T. Ulug, European Molecular Biology Laboratory, Postfach 10.2209, 6900 Heidelberg, F.R.G.

The transforming protein of polyoma virus, middle T antigen, forms a complex with, and is phosphorylated by, a cellular tyrosine kinase, pp60^{c-src}. Middle T binding increases the specific activity of pp60^{c-src}, apparently by altering the phosphorylation state of the enzyme. The kinase activity of pp60^{c-src} can also be activated by dephosphorylation, either in extracts or after purification of Tyr527 (the major tyrosine phosphorylation site). Our current model is that the activity of pp60^{c-src} is normally regulated by the opposing action of tyrosine kinases and phosphatases, and that middle T transformation is at least in part a result of constitutive rather than regulated pp60^{c-src} activation. Presently, we are concerned with defining the enzymes which regulate pp60^{c-src}, as well as the signal which normally activates it. Recent results will be presented.

We have recently discovered a third protein in the complex. This is a protein of 81 kD, which co-immunoprecipitates and co-sediments with both middle T and pp60^{c-src}. There is a correlation between the presence of p81 in the complex and phosphatidyl-inositol kinase activity in immunoprecipitates of middle T (an activity previously reported by Whitman *et al.* Nature; **315**, 239-242, 1985). It is therefore possible that middle T is able to bind to and cause the activation of both a tyrosine kinase and a phosphatidyl-inositol kinase.

S2.5

HUMAN ADENOVIRUS E1A STRUCTURE AND FUNCTION STUDIED THROUGH THE USE OF NOVEL INSERTION MUTANTS. B.S. Bautista [1], W.J. McCrory [1], F.L. Graham [1,2], Departments of [1]Biology and [2]Pathology, McMaster University, Hamilton, Ontario, Canada.

The E1a region of human adenovirus encodes two highly related proteins with a variety of different actions, notably ability to regulate the expression of viral and cellular genes and to immortalize primary cells. To map and study functionally important regions of E1a proteins we have developed a method for generating large numbers of insertion mutants throughout the E1a coding sequences. The method is based on insertion of a 39 bp synthetic oligonucleotide carrying the lac operator (binding site for lac repressor). Multicopy plasmids with the insert are easily identified by their ability to derepress host cell beta-galactosidase expression as a result of titration of the lac repressor. The synthetic lac operator can insert in two orientations, and is designed so that all reading frames are open in one orientation and closed in the other. In addition, it contains BamHI sites at both ends. Thus at each insertion site in E1a 3 mutants can be generated: one resulting in translation termination, one inserting a 13 aa oligopeptide into the E1a gene product, and a third, obtained by digestion with BamHI and ligation to "collapse" the insertion, resulting in a 2 aa insert. Mutations have been made throughout E1a and are being characterized in terms of their effects on E1a mediated trans-activation of cotransfected genes, on cell transformation, and on virus replication following rescue into full length viral genomes.

S2.6

THE INFLUENCE OF ADENOVIRUS TRANSFORMING GENES ON REGULATION OF CELL GROWTH
H.Th.M. Timmers, E.J.J. van Zoelen*, J.L.Bos, A.J. van der Eb Dept.Medical Biochemistry, University of Leiden, The Netherlands, Hubrecht Laboratory, Utrecht, The Netherlands.

The transforming and oncogenic potential of human adenoviruses is encoded by region E1, which consists of two transcriptional units, E1A and E1B. We have tested the hypothesis that transformation by adenoviruses is a result of an interaction of the viral gene products with the regulatory mechanisms of cell proliferation. Experiments on growth factor responsiveness have shown that non-oncogenic Ad5 E1-transformed rat cells are practically independent of growth factors from external sources for proliferation. Ad12 E1-transformed cells are independent of EGF and PDGF but require insulin or IGF-I for growth.

We have also studied the influence of Ad transformation on the expression of genes that are active in normal growing cells (c-myc, JE and KC have been tested). It was found that the expression of these genes is reduced 10-50-fold in both Ad5- and Ad12-transformed cells. Inhibition of the JE gene occurs at a post-transcriptional level. The results indicate that Ad region E1 (probably region E1A) can suppress the expression of a number of genes that normally appear to have functions in cell proliferation. This suggests that the E1A product(s) can functionally replace the activity of these genes in cell growth.

S3.1

GENETIC ORGANIZATION OF BUNYAVIRUSES AND ARENAVIRUSES. D.H.L. Bishop, NERC Institute of Virology, Oxford U.K.

The Bunyaviridae includes five virus genera: Bunyavirus, Hantavirus, Nairovirus, Phlebovirus and Uukuvirus. Viruses have a tripartite (L,M,S), single-stranded, negative-sense, RNA genome. Sequence and other analyses of bunyaviruses have shown that the viral nucleoprotein, N, and a non-structural protein, NS_S, are coded in overlapping reading frames in a single subgenomic, viral-complementary (ve) S mRNA species. The viral glycoproteins and NS_M, are coded as a polyprotein in subgenomic, veM mRNA. The viral transcriptase/replicase is coded in the veL mRNA species. Phleboviruses have a similar strategy of coding except that the phlebovirus S RNA has an ambisense coding arrangement, with N protein coded in a subgenomic veS mRNA species and NS_S protein coded in a subgenomic viral-sense mRNA species. The central intergenic region of the phlebovirus S RNA has the potential for secondary structure and is involved in mRNA transcription termination. Hantaviruses appear to code for a single gene product (N protein) in the veS RNA. The hantavirus veM RNA codes for the viral glycoproteins. No sequence information is available for nairoviruses. Uukuviruses appear to resemble phleboviruses in their overall coding strategies.

Arenaviruses have an ambisense S RNA with N protein coded in a veS mRNA and the glycoprotein precursor, GPC, coded by a subgenomic, viral-sense, S mRNA. The L RNA of arenaviruses codes for the viral transcriptase/replicase in its ve sequence.

S3.2

BIOGENESIS OF UUKUNIEMI VIRUS. R.F. Pettersson[1], N. Gahmberg[2], R. Rönnholm[1], L. Kääriäinen[2]. [1]Ludwig Institute for Cancer Research, Stockholm Branch, Sweden, and [2]Recombinant DNA Laboratory, University of Helsinki, Finland.

Uukuniemi virus is the prototype of the Uukuvirus genus within the Bunyaviridae family. We have studied the maturation of this virus with special reference to the biosynthesis of its two membrane glycoproteins G1 and G2 and the role of the Golgi complex (GC) in the maturation process. Previous work has shown that the virus matures in the GC. The glycoproteins accumulate in and cause a vacuolization and expansion of the GC. Using a ts mutant (ts12), we have shown that the morphological alterations of the GC are likely to be caused by the glycoproteins. To study the function of such an altered GC, we have followed the transport and glycosylation of Semliki Forest virus E1 membrane protein in double-infected cells. We found that E1 is efficiently transported to the plasma membrane, albeit with a 15-20 min delay. E1 is also terminally glycosylated (sialylated). The transport of collagen and fibronectin, two secretory proteins, also takes place normally. The result indicate that a morphologically altered GC, which has lost its normal stacked organization of the cisternae is able to transport, sort and terminally glycosylate plasma membrane and secretory proteins. We have determined the complete nucleotide sequence of the virion M RNA segment that codes for G1 and G2 and the results will be discussed.

S3.3

VIRULENCE DETERMINANTS OF CALIFORNIA ENCEPHALITIS VIRUSES

Francisco Gonzalez-Scarano, Barry Beatty, Daniel Sundin, Michael Endres, Neal Nathanson

The association of specific genes with virulence phenotypes in arboviruses requires the use of both vertebrate and invertebrate hosts, as well as non-mutagenized parent viruses. Four biological markers of the virulence of the California encephalitis viruses have been defined: mouse peripheral neuroinvasiveness, mouse neurovirulence, mosquito oral infectivity and mosquito intrathoracic infectivity. Virulence associated with each of theses routes of infections can be quantified. Using neuro-adapted strains, monoclonal antibody resistant variants and tissue culture adapted viruses we have assembled a panel of viruses that varies for each of these markers. Genetic reassortants show that all of the markers segregate with the middle RNA segment, indicating that its gene products are the most important in determining virulence in both of these hosts. Nevertheless, the results indicate the different loci within this segment determine each of these biologically important properties.

S3.4

PRELIMINARY EVALUATION OF A LIVE RECOMBINANT VIRUS VACCINE FOR LASSA FEVER. D. Auperin, J. Esposito, S. Fisher-Hoch, J. Lange, and J. McCormick. Division of Viral Diseases, Centers For Disease Control, Atlanta, Georgia, USA.

The ability to produce live recombinant viruses which express heterologous genes offers a new approach to the development of viral vaccines, as well as a means to explore the complex nature of the immunobiology of viral infection. Arenaviruses, which readily establish persistent infections in their natural rodent hosts and can cause severe and often fatal illness in humans, provide a rather unique theater for the application of this technology. A recombinant vaccinia virus expressing the Lassa virus glycoprotein gene has been constructed and evaluated in terms of its ability to ellicit a protective immune response in guinea pigs and rhesus monkeys. Qualitatively similar results were observed in both animal models. All vaccinated animals were protected from death, but did manifest varrying degrees of Lassa fever from which they completely recovered. The implications of these results as well as strategies for further modification and development of recombinant virus vaccines for Lassa fever will be discussed.

S3.5

PERSISTENT ARENAVIRUS INFECTION: ROLE OF VIRAL VARIANTS AND T CELL IMMUNITY. Rafi Ahmed, Microbiology & Immunology, UCLA School of Medicine, Los Angeles, CA 90024.

We have studied the mechanism of viral persistence and the potential of specific immune therapy to clear virus from a persistently infected host. Using mice infected with lymphocytic choriomeningitis virus (LCMV) as a model system, we have found that: 1) There is organ specific selection of viral mutants during chronic infection, and LCMV isolates with different biological properties are present in the central nervous system (CNS) and lymphoid tissues of carrier mice. 2) LCMV isolates from the CNS induce potent virus specific cytotoxic T cell (CTL) and delayed type hypersensitivity responses in adult mice and the infection is rapidly cleared. In contrast, LCMV isolates derived from the lymphoid tissues of carrier mice suppress virus specific T cell responses and cause persistent infections in adult mice. 3) The genetic change(s) that allow the variants to persist in adult mice and suppress T cell responses are located on the large (L) RNA segment. 4) Immune therapy of carrier mice with lymphoid cells from LCMV immune mice is effective in reducing both infectious virus and viral antigen. Clearance of this persistent and disseminated LCMV infection is mediated by virus specific MHC class I restricted $Lyt2^+$ T cells. In addition, there is a requirement for host derived bone marrow cells for effective viral clearance from the liver. 5) Both host and donor derived LCMV specific CTL responses can be recovered from carrier mice receiving immune therapy.

S3.6

Parameters influencing the balance between T cell mediated immunoprotection and immunopathology in LCMV-infections in mice

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Besides LCMV-viral genes determining tropism, replication, susceptibility to interferons and antibodies and other immune mechanisms, the dose and variability of the virus also influence the virus-host balance. In addition, various host factors seem to be important including T cell immunocompetence, antibody levels late during the infection, concomitant viral infections and the major histocompatibility gene complex (MHC). At least for the acute LCM-disease in mice that is triggered by intracerebrally injected LCMV and is mediated by cytotoxic T cells, susceptibility to disease can be shown to be determined by class I MHC antigens and correlated directly with the quality and quantity of the cytotoxic T cell response.

S4.1

TREATMENT OF CYTOMEGALOVIRUS INFECTION

Clyde Crumpacker, M.D. Department of Medicine
Beth Israel Hospital, Boston, MA

The acyclovir analogue (9-(1,3-dihydroxy-2-propoxymethyl) guanine (DHPG) is the first antiviral drug to be successfully employed in the treatment of serious, life-theatening CMV infection. This drug is activated to produce DHPG-triphosphate which is an effective inhibitor of the HCMV DNA polymerase. It is not firmly established whether activation to DHPG-TP occurs by cellular or viral enzymes.

In CMV retinitis, 90% of cases respond to 2 weeks intravenous DHPG with improved vision. CMV colitis and wasting syndrome in AIDS responds to DHPG treatment. Treatment of CMV pneumonia following bone marrow transplantation remains a challenge but in a collaborative study at several medical centers 9 of 21 CMV pneumonia cases survived with DHPG treatment. Following treatment with DHPG a high rate of relapses occur and patients require IV maintenance therapy at 30 mg/Kg/week to suppress relapse of serious CMV disease. The main side effect of DHPG treatment is a reversable neutropenia in 30% of cases. DHPG represents an important first step in successful treatment of CMV disease.

S4.2

ANTIVIRAL THERAPY OF VARICELLA ZOSTER INFECTIONS.

R.J. Whitley, University of Alabama, Birmingham, AL, USA

The availability of acyclovir and vidarabine has led to significant improvement in the management of varicella-zoster virus infections in the immunocompromised host. Controlled clinical studies utilizing these medications when given intravenously to high-risk patient populations, namely the immunocompromised host, will be summarized. While the parenteral administration of vidarabine accelerates healing of cutaneous lesions, and decreases dissemination, pain and visceral complications, recent data indicate superiority of acyclovir. Data available from the National Institutes of Allergy and Infectious Diseases Collaborative Antiviral Study Group identify patients at significant risk for more severe disease. These factors include type of malignancy, disease duration, and immune adherence in antibody titers at presentation. These data can be utilized to define patients for whom ambulatory care therapy with oral medications appear reasonable.

S4.3

Antiviral Therapy of Human Immunodeficiency Virus. M. S. Hirsch, Department of Medicine, Massachusetts General Hospital, Harvard Medical School, Boston, MA USA.

The AIDS epidemic poses a major threat to our population. Urgently needed are safe and effective antiviral agents to treat individuals infected with HIV, the etiologic agent. The elucidation of viral replicative mechanisms has allowed the development and testing of several agents active against HIV in vitro. Inhibitors of reverse transcriptase that demonstrate activity include azidothymidine (AZT), phosphonoformate (PFA), antimoniotungstate (HPA-23), and dideoxycytidine (ddC). AZT has demonstrated clinical benefit in patients who have recovered from Pneumocystis carinii pneumonia or who have advanced AIDS-related complex with T-helper lymphocytes in peripheral blood less than 200/cumm. Ribavirin and interferons alpha, beta, and gamma also inhibit HIV replication, although their mechanisms of action are less clear.

Combinations of anti-HIV agents have also been evaluated in vitro, and are now being studied in patients. In vitro synergistic antiviral activity has been demonstrated between AZT and interferon alpha, PFA and interferon alpha, PFA and ribavirin, ddC and interferon alpha. In contrast, antagonism occurs when combinations of AZT and ribavirin are employed.

Clinical trials of AZT, ribavirin and ddC are underway.

S4.4

RIBAVIRIN AND ITS CURRENT PLACE IN ANTIVIRAL THERAPY. B.E. Gilbert. Department of Microbiology and Immunology, Baylor College of Medicine, Houston, Texas, USA.

Ribavirin is a purine nucleoside analog exhibiting in vitro antiviral activity against a wide variety of common and exotic viruses. Ribavirin is metabolized intracellularly to its 5'-phosphate derivatives which directly or indirectly inhibit viral replication and in man is rapidly excreted in the urine unchanged or as the deribosylated product. Ribavirin administered to humans as a small particle aerosol is rapidly deposited throughout the respiratory tract and attains concentration that are inhibitory to both influenza A and B viruses and RSV. Ribavirin aerosol treatment of respiratory syncytial virus infection in infants has recently been approved in the United States and Canada. In clinical trials ribavirin aerosol also has been effective in the treatment of influenza A and B virus infections and is being evaluated for its effectiveness against human immunodeficiency virus infection. Oral ribavirin appears to be effective in the treatment of acute hepatitis A infections, measles and Lassa fever. Although transient anemias and elevated bilirubin levels have been reported for oral or intravenous administration of ribavirin, these side effects have not been observed during small particle aerosol treatment. We feel that administration of ribavirin by small particle for the treatment of viral respiratory infections opens new avenues for increasing effectiveness while decreasing toxicity.

S4.5

APPROACHES TO THE DEVELOPMENT OF NEW ANTIVIRAL DRUGS.

G. Darby, K. L. Powell, D. J. Purifoy, B. A. Larder. Department of Biochemical Virology, The Wellcome Research Laboratories, Beckenham, Kent, U.K.

Antiviral drug discovery has entered an exciting new era in which our knowledge of virus infection processes has advanced to the point where we can identify specific target proteins encoded by viruses, and where modern technology has provided the tools for rational drug design. The starting point is the identification of an essential virus gene product which interacts with a small defined chemical moiety such as an enzyme substrate or surface receptor on a cell. Such an interaction provides the focal point for the design process which may then progress down any one of several alternative routes. The objective is to create small chemical entities which are able to block the interaction and thus interfere with protein function. Although such molecules can be based on the structures of substrates or receptors, the whole process enters a new dimension if the detailed structure of the protein and its interactions are known.

The above approach will be illustrated by our work on the reverse transcriptase of the AIDS virus HIV.

S5.1

CO-TRANSLATIONAL DISASSEMBLY OF FILAMENTOUS VIRUS AND PSEUDOVIRUS PARTICLES IN VITRO AND IN VIVO. T.M.A.Wilson[1], J.G.Shaw[2], D.R. Gallie[1], D.E. Sleat[1], K.A. Plaskitt[3], J.W.Watts[3]. Dept. of Virus Research[1] and Cell Biology[3], John Innes Institute, Colney Lane, Norwich NR4 7UH, U.K.; Dept. of Plant Pathology[2], University of Kentucky, Lexington, Kentucky 40546, U.S.A.

Tobacco mosaic virus (TMV) RNA may be released from its protective ribonucleocapsid by co-translational disassembly in vivo. In vitro, 70S and 80S ribosomes can uncoat the 5'-cistron(s) of TMV RNA while synthesizing polypeptide(s) of 126K [and 183K]. Distinctive intermediate translation complexes, "striposomes", have been characterised in vitro and detected in vivo, in TMV-inoculated tobacco or pea epidermis. Complexes with similar physicochemical properties have been isolated from tobacco inoculated with other filamentous plant viruses.

To examine the site and mechanism of nucleocapsid disassembly, polyclonal antibodies, raised against the 126K protein, were used to immunogold-label striposomes in situ or in extracts of inoculated cells or protoplasts. A family of SP6-transcription plasmids was developed, each contained a 5'-proximal gene for a protein alien to plant cells and a correctly-oriented origin-of-assembly sequence from TMV RNA. Run-off, in vitro transcripts were packaged into "pseudovirus" particles resembling TMV and used to "pseudo-infect" protoplasts or cells from various sources. These artificial nucleocapsids can be used to study the earliest events of plant virus-cell interaction, without the added complexity of replication.

S5.2

INTERACTION BETWEEN COWPEA CHLOROTIC MOTTLE VIRUS AND COWPEA PROTOPLASTS: PENETRATION AND UNCOATING. B.J.M. Verduin, J.W. Roenhorst. Department of Virology, Agricultural University, Wageningen, the Netherlands.

Binding/penetration was measured as virus associated with protoplasts after incubation with ^{35}S-labelled CCMV and appropriate wash. At 0°C and 25°C the amount of virus associated with protoplasts increased with the amount of virus added. An input of $25*10^8$ virusparticles per protoplast resulted in binding of 4,000 and 14,000 particles per protoplast respectively. In the presence of polyethylene glycol (PEG, Mr 6000) an increase to 80,000 associated particles occurred at both temperatures. In the latter case ca. 50% of the protoplasts became infected, as determined by immunofluorescence 16 h after inoculation. No infection occurred in the absence of PEG.

Variation of pH or ionic strength in the absence of PEG caused hardly any change in binding and again there was no infection. In the presence of PEG increase of pH resulted in lower and increase in ionic strength in higher binding numbers. The percentage of infected protoplasts was not influenced by pH, and increased with ionic strength to 70% at 0.35 M NaCl.

These data support the model of entry where virus is introduced into the cytoplasm through lesions in the plasma membrane, followed by cotranslational disassembly, already demonstrated for partially swollen CCMV in vitro.

S5.3

RESISTANCE TO TMV INFECTION IN TRANSGENIC PLANTS THAT EXPRESS A TMV-COAT PROTEIN GENE. R.N. Beachy, R.E. Nelson, P. Powell Abel, J.A. Register III, S. McCormick*, R.T. Fraley*. Department of Biology, Washington University, St. Louis, MO, 63130, U.S.A.; *Monsanto Company, St. Louis, MO, 63198, U.S.A.

A chimeric gene that caused the constitutive and high level expression of a gene encoding the coat protein (CP) of the U_1 strain of tobacco mosaic virus (TMV) was introduced into tobacco and tomato plants on a disarmed Ti-plasmid in Agrobacterium tumefaciens. Transgenic plants and their progeny were protected against infection by the U_1 and other strains of TMV and tomato mosaic virus. Protection was recorded as reduction in the number of sites where infection is established, and as reduced virus spread following infection. Protection was considerably less effective against TMV-RNA than against virions. Protection was also expressed in protoplasts isolated from the transgenic plants that express the CP-gene. It is proposed that protection results from the blockage of an early event in the infection, for example, uncoating of the virion, or translation of the virion RNA (but not unencapsidated viral RNA).

Expression of other viral sequences in transgenic plants did not substantively interfere with virus infection.

S5.4

REPRESSED VIRUS REPLICATION AND SYMPTOM EXPRESSION IN PLANTS TRANSFORMED WITH VIRUS SATELLITE NUCLEIC ACID. B.D. Harrison[1], M.A. Mayo[1], D.J. Robinson[1], D.C. Baulcombe[2]. Scottish Crop Research Institute[1], Invergowrie, Dundee and Plant Breeding Institute[2], Trumpington, Cambridge, UK.

Tests were made to analyse the reaction to virus infection of tobacco plants transformed, using the Agrobacterium Ti plasmid system, with DNA copies of a benign satellite RNA of cucumber mosaic cucumovirus (CMV). When these plants were infected with a satellite-free isolate of CMV, large amounts of unit-length satellite RNA were produced, the accumulation of CMV genomic RNA and of CMV particle protein in systemically infected leaves were repressed and systemic symptoms were milder than those in infected non-transformed plants. The ability of transformed plants to react in this way was transmitted to F1 progeny seedlings. The satellite RNA produced in transformed plants was packaged in virus particles and became a transmissible component of the virus culture, which was less virulent as a result.

Similar effects were observed when transformed plants were infected with tomato asperny cucumovirus but not when they were infected with peanut stunt cucumovirus or viruses belonging to several other groups. This type of genetically engineered virus resistance has promise as a basis for a novel method of virus control.

S5.5

TOBACCO MOSAIC VIRUS - CHLOROPLAST ASSOCIATIONS. Milton Zaitlin, Ralf Dietzgen, David D. Dunigan and James E. Schoelz. Department of Plant Pathology, Cornell University, Ithaca, USA.

Small amounts of viral coded components have been found in chloroplasts isolated from tobacco mosaic virus (TMV)-infected plants. TMV RNA, coat protein (Reinero and Beachy, 1986, *Plant Molecular Biology* 6:291), pseudovirions (Rochon and Siegel, 1984, *PNAS USA* 81:1719), and H protein (Collmer *et al.*, 1986, *Virology* 126:429) are found there. H protein has been shown to be an unusual fusion protein composed of a backbone of the viral coat protein coupled to a protein of host origin through a postulated isopeptide linkage. The means whereby these TMV components either enter or are synthesized in chloroplasts and their possible roles in pathogenesis are being investigated.

S5.6

THE BIOCHEMISTRY OF HOST RESPONSE TO TOBACCO MOSAIC VIRUS R. S. S. Fraser, R. J. Whenham, A. Gerwitz. Plant Science Division, Institute of Horticultural Research, Wellesbourne, Warwick CV35 9EF, U.K.

Infection of tobacco or tomato plants with tobacco mosaic virus (TMV) inhibits growth and causes leaf mosaic and distortion. Quantitative interactions between virus multiplication and growth inhibition have provided evidence for competitive inhibition of growth at high virus concentrations.

Growth has also been shown to be inhibited by virus-stimulated increase in the concentration of the plant growth regulator abscisic acid (ABA), in tobacco but not in tomato. The effects of TMV infection on ABA metabolism in tobacco have been studied in detail: the virus causes a change in synthesis and in compartmentation. This is at least partly responsible for the formation of dark green areas of mosaic.

Cytokinin metabolism has also been examined in TMV-infected tobacco, using a new, highly selective and sensitive gas chromatographic assay method. Infection decreased zeatin and zeatin riboside concentrations in dark green areas, but increased zeatin-O-glucoside, suggesting an increased rate of cytokinin metabolism.

Possible involvement of altered growth regulator metabolism in the resistance of dark green tissue to virus invasion will be discussed.

S6.1

A new rhabdovirus isolated from cultured hirame (Japanese flounder, Paralichthys olivaceus) and ayu (Plecoglossus altivelis) in Japan. T. Kimura, M. Yoshimizu, T. Nishizawa and N. Ohseko. Laboratory of Microbiology, Faculty of Fisheries, Hokkaido University, Hakodate, Japan.

In 1984, a new rhabdovirus was isolated from moribund cultured hirame, and ayu fry. At temperatures between 5 to 20°C the virus replicated and induced cytophathic effects which progressed to eventual cytolysis, in susceptible cell lines, including FHM, EPC, BF-2, RTG-2, STE-137, HF-1, BB, YNK, CCO and EK-1. The CHSE-214, KO-6 and CHH-1 cell lines were refractry. Maximum infectivity of culture grown virus was about $10^{9.0}$-$10^{9.5}$ $TCID_{50}$/ml in FHM or EPC cell lines, and virus replicated optimally at 15 to 20°C.

Virus particles were bullet shaped, 80 x 180 to 200 nm and have 5 structure proteins. M.W. of N protein was different from that of IHNV. Infectivity was not reduced by antisera against IHNV, VHSV, SVCV, PFRV, EVA and EVX. The viral isolate was pathogenic for hirame and rainbow trout (Salmo gairdneri), black sea bream (Mylio macrocephalus) and red sea bream (Chrysophrys major). Artificially infected hirame and rainbow trout showed the hemorrhage in muscles and the kidney tissue was necrotic, these signs of infection in hirame were similar to those of natural outbreaks in this fish. HRV is now considered to be important pathogen of cultured hirame and salmonid in Japan.

S6.2

THE PIKE AS A CENTRAL HOST IN VHS

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Federal Research Centre for Virus Diseases of Animals, P.O.B. 1149, D-7400 Tübingen, FRG.

The rainbow trout (Salmo gairdneri) in aquaculture is assumed to be the main host for viral haemorrhagic septicaemia virus. Heavy losses occur with that species. Nevertheless it was reported that other fish species may also be susceptible for that pathogen, but outbreaks in wild living animals were never described. In a field study it could be shown that brown trout (Salmo trutta fario), pike (Esox lucius) and whitefish (Coregonus spec.) in wild waters revealed antibodies against VHS-virus to a high degree. Prevalence of antibodies in brown trout was found in the southern part of Germany as high as 67%. The highest prevalence in pike was found to be 68%. In white fish from the Lake of Constance prevalence of antibodies was 35%.

The inapparent infection in brown trout populations vanished within 3 years. In contrast, the infection of pike persisted in several populations for years (usually infected pike show severe symptoms of VHS). The course of infection in white fish could not be followed up for a longer period.

From the field study it was assumed that brown trout in wild waters and rainbow trout in aquaculture were infected by the pike. The origin of infection in the white fish is not clear yet. It is supposed that the pike play the main role in the epizootiology of VHS.

S6.3

DEVELOPMENT OF A VACCINE TO IHN VIRUS BY MOLECULAR CLONING. J.C. Leong, R. Gilmore, J. Feyereisen-Koener, G. Kurath, N.M. Engelking, and C. Passavant. Department of Microbiology, Oregon State University, Corvallis, Oregon 97331-1906, U.S.A

Infectious hematopoietic necrosis virus (IHNV), a fish rhabdovirus, is a major pathogen in salmon and trout and the only available method of controlling the disease in hatcheries has been the destruction of carriers or infected fish and eggs. Since the virus is transmitted via water and wild fish reside in neighboring waters, the administration of an attenuated viral vaccine was not feasible without costly trials and assurances of nonvirulence in all species. Thus, the development of a subunit vaccine to IHNV was initiated using recombinant DNA techniques.

Complementary DNA clones to the six IHNV mRNA species were constructed and a complete copy of the viral glycoprotein gene was selected by in vitro translation and immunoblot analysis of RNA transcribed from a glycoprotein plasmid. Portions of the glycoprotein gene were subcloned in pATH expression vectors under a TrpE promoter. The expression of the glycoprotein gene was determined by colony immunoblots. The expressed protein was used to immunize fish and preliminary trials indicated that the bacterially produced viral glycoprotein induces protective immunity.

S6.4

THE EMERGENCE OF A MARINE VIRUS. A.W. Smith, E.S. Berry, D.E. Skilling, College of Veterinary Medicine, Oregon State University, Corvallis, Oregon, USA

Fifty-five years ago a new virus disease said to be host specific for swine appeared along the California coastal zones. This virus produced vesicles on the feet and mouths of swine and was associated with their eating raw garbage. Through the medium of raw garbage the virus was eventually spread throughout the USA until it was successfully eradicated some 24 years after its first appearance. The original source of the virus which contaminated the garbage was unknown.

Forty years later (1972) the first virus isolated from pinnipeds (California sea lions, Zalophus californianus) proved to be indistinguishable from this agent and was classified as a calicivirus called San Miguel Sea Lion Virus (SMSV). In fact, SMSV will cause a vesicular disease in swine and will spread by contact. By 1987, caliciviruses exhibiting these same characteristics have been isolated from 6 species of marine mammals, one species of ocean fish, a series of terrestrial animals, and one human, a calicivirus researcher.

Today, this virus of ocean origin exhibits extensive antigenic variability, an unusually broad host range, and is emerging from its ocean reservoirs to infect a widening variety of terrestrial animal hosts.

S6.5

CHARACTERISTICS OF A NEW REOVIRUS FROM COHO SALMON (ONCORHYNCHUS KISUTCH). J.R. Winton and C.N. Lannan. U.S. Fish and Wildlife Service, National Fishery Research Center, Bldg 204 Naval Station, Seattle, USA and Oregon State University, Marine Science Center, Newport, USA.

In December of 1985 we isolated a virus from kidney and spleen tissues of adult coho salmon returning to the Coquille River on the coast of Oregon, USA. This virus replicated optimally at 18°C in monolayer cell cultures derived from Pacific salmon. Cytopathic effect in these cells consisted of plaque-like syncytia of cytoplasmic fusion. The virions were non-enveloped particles, 70 nm in diameter, composed of a double capsid shell. The virus genome contained 11 segments of dsRNA distributed among three size classes. Intraperitoneal injection of the virus into juvenile coho salmon and rainbow trout (Salmo gairdneri) did not result in mortality.

In recent years, several members of the Reoviridae have been isolated from aquatic animals. These viruses, usually recovered from warm-water fishes, seem to form a novel genus of the family. Analysis of the coho salmon reovirus genome using 9% SDS-PAGE gels showed that the virus had an electropherotype distinct from the other viruses of this group. The virus was serologically unrelated to the chum salmon virus, the only other reovirus isolated from a salmonid fish.

S6.6

HERPESVIRUS CYPRINI: PATHOGENICITY AND ONCOGENICITY. T. Sano, N. Shima and M. Akimoto. Department of Aquaculture, Tokyo University of Fisheries, Tokyo, Japan

Herpesvirus cyprini (CHV) revealed lethal pathogenicity to carp fry raised for 4 wk at 16-20°C after 1h immersion inoculation by $10^{3.7}$-4.2 $TCID_{50}$/ml of CHV contained in one l water at 20°C: the cumulative mortality for 2 wk old fry was 86-97% in common carp, 93% in fancy carp: 20% in 1 mo old fancy carp: 0% in the both 2 mo old common and fancy carp. It did not produce mortality in crucian carp, grass carp and willow shiner frys. CHV revealed oncogenicity to carp: the papilloma reproduction was recognized at the incidence of 55-66% to both the carp frys which had survived in the infectivity trials 5 to 6 mo after 1h immersion inoculation by the virus. This virus was reisolated from all moribund fish and all survivors. While the specific fluorescein emission was found more frequently in the internal organs such as the liver, kidney etc. in 2 wk old fry, it was found in greater concentrations in the gill epidermis in 4 wk old fry. CHV also reproduced the papilloma at the incidence of 13% to adult mirror carp and at 10% to adult fancy carp 5 mo after IP inoculation of 0.5-1ml of 10^{5} $TCID_{50}$/ml per fish. The tumor formation located on the pectoral fin, caudal fin, trunk skin, mandible and IP inoculation site.

The virus was reisolated only from the tissue of reproduced papilloma.

S7.1

HEPATITIS A VIRUS. I.D. Gust, Virus Laboratory, Fairfield Hospital for Communicable Diseases, Melbourne, Australia.

Hepatitis A is a common virus infection which is caused by a picornavirus. The aetiological agent is currently classified as an enterovirus, but has a number of physical features suggesting that it differs from other members of this group.

The development of sensitive diagnostic tests has provided new insights into the epidemiology and natural history of the disease and has demonstrated that the disease is a major public health problem in developing countries.

Recently, much has been learnt about the molecular biology of the virus and attempts are underway to develop both killed and live attenuated vaccines. These developments will be described in detail.

S7.2

TWO DIFFERENT MECHANISMS FOR HEPATITIS B VIRUS INDUCED HEPATOCELLULAR CARCINOMA. Pierre Tiollais, Tarik Möröy, Agnès Marchio, Jeanne Etiemble, Hugues de Thé, Marie-Annick Buendia, and Anne Dejean. Unité de Recombinaison et Expression Génétique (INSERM U.163, CNRS UA.271), Institut Pasteur, 28 rue du Docteur Roux - 75724 Paris Cédex 15, France.

Hepatitis B virus was found to contribute in at least two possible different manners to the development of hepatocellular carcinoma : in a direct way by inserting in an exon from a human gene, that we referred to as hap, which shows a strong homology with the DNA-binding domain of h-c-erbA and the steroid receptor genes. The HBV integration could have resulted in the overexpression of a truncated hap protein and could thus have directly participated to the hepatocyte transformation. On the other hand, rearrangements by chromosomal translocation of the c-myc proto-oncogene found in several liver tumors of WHV-infected woodchucks have resulted in an overexpression of the c-myc gene. Viral sequences were integrated in the genomic DNA of these tumors, but not in the vicinity of the altered c-myc locus excluding a direct role of the virus in the oncogene activation. c-myc rearrangements could have been favoured by chromosomal instability due to WHV integration into the cellular DNA.

S7.3

HEPATITIS DELTA VIRUS (DELTA AGENT). J.L. Gerin, Division of Molecular Virology and Immunology, Georgetown University Medical Center, Rockville, MD, U.S.A.

The hepatitis delta virus (HDV) is defective and requires active hepatitis B virus (HBV) infection for its replication in man. HDV is pathogenic a typically causes severe acute and chronic liver disease, Type D or delta hepatitis. HDV is a 36 nanometer particle that consists of virus-specific antigen (HDAg) and a small RNA genome both of which are encoated by the surface antigen of the host hepadnavirus infection. The 1.7 kb RNA genome is poly A minus, single-stranded and circular. Cloned cDNA fragment spanning the entire 1679 base genome have been used to detect and quantitate HDV RNA in clinical specimens and permit detailed examination of the genomic structure and organization. Open reading frames (ORF) exist on both strands of the cloned cDNA; an ORF in the antigenomic strand encodes at least a portion of the two related 24 kd and 27 kd structural polypeptides of HDAg. While virion RNA has a single polarity, both genomic and antigenomic species are found in infected liver tissue. The analysis of these forms indicates that HDV replicates in a manner consistent with models proposed for the replication of certain viriods of higher plants. HDV appears to be unique among the known animal viruses. Knowledge of HDV structure and replication may permit the identification of the HBV helper functions and the development of effective control strategies for prevention and treatment of delta hepatitis.

S7.4

BLOOD-BORNE NON-A, NON-B, HEPATITIS VIRUS. D. Bradley, Centers for Disease Control, Atlanta, GA, USA

ABSTRACT NOT AVAILABLE

S7.5

WATER-BORNE NON-A, NON-B HEPATITIS VIRUS. M. Balayan, Institute of Poliomyelitis and Viral Encephalitides, Moscow, USSR

ABSTRACT NOT AVAILABLE

S8.1

DELINEATION OF VIRAL EPITOPES BY SOLID-PHASE IMMUNOASSAYS : ADVANTAGES AND PITFALLS. I. Dore, D. Altschuh, E. Weiss, C. Porta, M.H.V. Van Regenmortel, Institut de Biologie Moléculaire et Cellulaire, CNRS, 15 rue Descartes 67084 Strasbourg-Cédex, France.

Of 30 McAbs raised against tobacco mosaic virus protein (TMVP), 5 did not react with virions in ELISA using antibody-coated plates ; they were specific for cryptotopes and recognized synthetic peptides of 13-28 residues. The location of the epitopes recognized by these 5 McAbs was further defined by comparing common accessible surfaces in TMV mutants and related viruses, using a probe of 7 A radius. The delineation of epitopes was also carried out by competitive ELISA using antipeptide antisera and labelled McAbs.

Of 18 McAbs raised against TMV, 8 did not react with viral subunits in ELISA and were specific for neotopes. These McAbs also did not bind to the virions in an ELISA using antigen-coated plates. One explanation proposed previously for this finding was that the virions were degraded by adsorption to the solid phase ; it is indeed widely recognized that proteins are partly denatured on solid-phases. This phenomenon was analysed by performing ELISA experiments on electron microscope grids and controlling the presence of the different types of epitopes using antibodies labelled with colloidal gold. It was found that at pH 9.6, viral subunits were preferentially bound to the solid phase and interfered with adsorption of virions.

S8.2

MOLECULAR IMMUNOLOGICAL ASPECTS OF FOOT-AND MOUTH DISEASE VIRUS. E. Pfaff, H-J. Thiel*, H. Schaller, Microbiology & Center for Molecular Biology, University of Heidelberg, Heidelberg, FRG; *Federal Research Institute for Animal Diseases, Tübingen, FRG.

The major antigenic determinant of foot-and mouth disease virus has been located within the region from amino acid 144 to 159 on coat protein VP1*. A synthetic peptide representing this region can protect guinea pigs against viral infection. Immuno affinity chromatography using this synthetic peptide eliminates >90% of the neutralising activity of a hyper-immune serum. To investigate the antigenic determinant in more detail, neutralising monoclonal antibodies (McAb) were raised against complete virus and against the synthetic peptide. These McAbs could be divided into 4 neutralisation groups. The binding sites of the McAbs were localized by different methods e.g. Western blot, Elisa and competition experiments. 2 of the groups of McAbs bound to the synthetic peptide, isolated VP1 and viral particles and binding could be inhibited completely by the peptide. The McAbs of the two other groups bound only to complete viral particles and weakly to the peptide.

To correlate the different neutralisation pattern with the primary structure of the coat protein VP1, the nucleotide sequences coding for the antibody binding sites were determined for all FMDV strains tested. In conclusion this analysis demonstrated that McAbs from the first two groups recognize overlapping sequential epitopes, whereas the McAbs from the two other groups recognize conformational epitopes.

To support these findings, escaped mutants were selected from all McAb groups and sequenced. The results obtained showed amino acid exchanges in the antibody binding site in VP1 in the case of the McAbs which recognize the sequential epitopes. In contrast, in the other cases, amino acid exchanges were only found in the two other coat proteins VP2 and VP3, which by themselves do not induce neutralising antibodies.

*Pfaff, E.; Mussgay, M.; Boehm, H.O.; Schulz, G.E.; Schaller, H. (1982) EMBO J. 1, 869-874

S8.3

ANTIGENIC STRUCTURE AND VARIATION IN PARAMYXOVIRUSES. C.Örvell, M.A.Mufson, R.Rydbeck, A.Löve, H.Sheshberadaran and E.Norrby. Department of virology, National Bacteriological Laboratory and Karolinska Institute, School of Medicine, Stockholm, Sweden and Department of Medicine, Marshall University, Huntington, West Virginia.

Intratypic antigenic variation of five different paramyxoviruses, mumps, parainfluenza type 3 virus, measles, canine distemper and RS virus has been studied in our laboratory, exemplified by mumps.virus and RS virus. Mumps virus strains varied in the HN and NP protein, whereas little variation was observed in the F, P and M proteins. RS virus formed a special situation in comparison to the other viruses, in as much as two clearly defined subgroups have been observed with human RS virus strains.

Monoclonal antibodies that react with several viral strains may be used to define common immunogenic domains on the corresponding structural protein. These monoclonal antibodies may be reacted with overlapping synthetic peptides representing the entire protein. If the monoclonal antibody and also human convalescent sera react with a particular segment, this synthetic peptide has a potential value as antigen in diagnostic tests. In the same way monoclonal antibodies with neutralizing and/or protective activity may be used to define immunologic domains of importance for immunity to the virus. This knowledge may open the way for synthetic, subcomponent vaccines in the future.

S8.4

EPITOPE MAPPING OF HERPES SIMPLEX VIRUS GLYCOPROTEIN D BY SITE-DIRECTED MUTAGENESIS. G.H. Cohen, W. Wilcox, M.I. Muggeridge, D.A. Long, D. Sodora and R.J. Eisenberg. University of Pennsylvania, Philadelphia, Pa. 19104

Herpes simplex viruses (HSVs) are enveloped DNA viruses that cause human diseases, including cold sores, eye and genital infections, and encephalitis. Glycoprotein D (gD) of HSV is a structural component of the virion envelope and is a target of the host immune response. Studies with monoclonal antibodies (MCAb) have suggested that gD plays an important role in the initial stages of viral infection, i.e., adsorption and cell fusion. Our goal has been to define antigenic domains of gD, and now that we have constructed a detailed epitope map, we are interested in relating this knowledge to the biological properties of the protein. Our current approach is to construct gD mutants, using the cloned gene, and analyze the effects of the mutations on antigenicity and function. We have constructed a plasmid that permits expression of gD-1 protein in mammalian cells, developed a variety of deletion and point mutants and have studied their properties. Our results show that the antigenic structure of gD is maintained by disulfide bonds. Of the seven cysteine residues in the protein, six are critical for formation of discontinuous epitopes. Alteration of any one of these six cysteines has profound effects on antigenicity and processing of gD. Analysis of deletion and MCAb resistant (mar) mutants has enabled us to fine map the major neutralizing site on gD.

S8.5

THE CRYSTAL STRUCTURE OF ANTIBODIES COMPLEXED WITH INFLUENZA VIRUS NEURAMINIDASE (NA). W.G. Laver, P.M. Colman, R.G. Webster & G.M. Air, John Curtin School of Medical Research Canberra, Melbourne, Memphis and Birmingham, Alabama.

We have grown crystals of monoclonal antibody Fab fragments complexed with influenza virus neuraminidase of the N9 subtype. Two such crystalline complexes, which diffract x-rays to beyond 3Å, have been obtained. In each case, four Fab fragments are bound to the NA tetramer. The two antibodies, NC41 and NC10 recognize overlapping epitopes on the NA.

The structure of the NC41 Fab-N9 NA complex has been solved and shows an epitope involving 4 surface loops of the neuraminidase. Sequence changes in these loops abolish or diminish antibody binding. The structure also reveals an unusual pairing pattern between the domains of light and heavy chains in the variable module of the antibody. This implies that association with antigen can induce changes in the quaternary structure of the Fab, through a sliding of domains at the VL-VH interface.

In addition, Fab binding has altered the conformation of one of the surface loops of the antigen, distorting the position of an active-site arginine (371) and providing an explanation for the low neuraminidase activity of this complex in which the active centre remains accessible to small subtrates.

S8.6

PERSISTENT FRACTION IN PICORNAVIRUSES: A PENTAMER-BRIDGING HYPOTHESIS. Roland Rueckert, Anne Mosser and Donna Leippe. Biophysics Laboratory, University of Wisconsin, Madison, WI 53706, USA.

We have studied the neutralization profiles of 42 monoclonal antibodies representing 7 target sites whose locations on the surfaces of type 1 poliovirus (PV1) and human rhinovirus 14 are known. The results show a strong correlation between separation of target sites (related by a 2-fold symmetry axis on the virus) and persistent fraction (PF), i.e. surviving infectivity with saturating amount of antibody. Thus 11/22 of the IgGs directed against sites separated by 9 to 12 nm were PF-minus ($PF \ll 10^{-3}$). All 23 IgGs directed against sites separated by only 5-6 nm neutralisers were PF-plus ($PF = 10^{-2} - 10^{-3}$).

We propose that the persistent fraction of infectivity in immune complexes is due to inability of antibody to establish bivalent bridges between neighboring pentamers; i.e. complete neutralization is accomplished by crosslinking pentamers. The bridging hypothesis predicts: (1) that the "minimal spanning range" for IgG lies somewhere between 8 and 9 nm; (2) and that some antibodies against sites within the spanning range bind outside the permissible "scanning angle," i.e. the first leg of the antibody anchors at such an orientation that its second leg is unable to reach its partner site. It may be possible to test the scanning-angle hypothesis by crystallographic analysis of complexes betwen antibody and its target site.

S9.1

AFRICAN SWINE FEVER (ASF) VIRUS: A 'MISSING' LINK BETWEEN POXVIRUSES AND IRIDOVIRUSES? E. Viñuela, M.L.Salas, A. Talavera, J.M. Almendral, A.L. Carrascosa, A. Gonsáles, R. Blasco, C.L.Otín, A. Alcamí, M. del Val, F. Almasán, J. Salas, M. Agüero, Centro de Biología Molecular, Facultad de Ciencias,Universidad Autónoma, Canto Blanco, 28049 Madrid, Spain

ASF virus particles are icosahedrals of about 200 nm that consist of an 80 nm core surrounded by a lipoprotein envelope. The virus contains 30-40 proteins, which include many enzymes able to synthesize, cap, methylate and polyadenylate functional messenger RNA. The ASF virus genome is a noninfectious double-stranded DNA molecule of about 170,000 base pairs. At both termini the two DNA strands are covalently linked by a partially base-paired hairpin loop into a single polynucleotide chain. The virus particle can synthesize in vitro functional virus mRNAs in the absence of protein biosynthesis. One of the major biological problems posed by ASF virus-infected animals is that the virus-specific antibodies are not neutralizing. The presence in ASF virus DNA of several multigene families, a finding not reported for any other virus, may be related to the ability of ASF virus to evade the immune system.

S9.2

REPLICATION CYCLE OF FROG VIRUS 3. R. Goorha, Department of Virology and Molecular Biology, St. Jude Children's Research Hospital, Memphis, Tennessee 38101, USA.

Frog virus 3, a member of the Iridoviridae family, is a large icosahedral virus. The FV3 genome is circularly permuted and terminally redundant. The viral DNA replicates in two physically separated stages, the first involving origin-specific DNA replication in the nucleus and the second involving recombination/replication in the cytoplasm. About 20% of the cytosine residues in FV3 DNA are methylated. Methylation of DNA protects the genome from an FV3-specific endonuclease and thus functions in a manner analogous to restriction-modification system of bacteria.

FV3 transcription in the infected cell is sequentially ordered and coordinately regulated to produce at least three different classes of mRNA. Additionally, quantitative controls are superimposed on all three classes of FV3 mRNAs. A unique feature of FV3 transcription is that a trans-activating FV3 protein endows host RNA polymerase II an ability to transcribe methylated genome. FV3 gene expression is also regulated at the post-transcriptional level. A viral protein inhibits the translation of early messages late in infection whereas another regulatory protein is responsible for the turn-on of late mRNA translation. The study of the replication cycle of FV3 has led to a deepened appreciation of mechanisms of genome replication and gene expression employed by animal viruses.

S9.3

POXVIRUS VIRULENCE R. Mark L. Buller Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892 USA.

The severity of a natural poxvirus-induced disease is dependent on a number of virus gene products which enhance the ability of the virus to succeed in an environment in which both learned and innate host defense mechanisms are mobilized to respond to the infection. In order to study the relative importance of different genes in virus virulence, a virus-host model must be chosen in which the disease is severe with high mortality as with ectromelia virus infection of certain strains of inbred mice. Furthermore, methods must be available for constructing and isolating virus mutants in single genes, and for detecting alterations in virus pathogenesis. Using ectromelia, cowpox, and vaccinia viruses in mice, rabbits, and fertilized chicken eggs, unique virus virulence and host range functions have been characterized in a number of laboratories. This presentation will examine known classes of virus virulence and host-range functions, and speculate on the existence of other as yet undiscovered virulence factors.

S9.4

GENETIC ANALYSIS OF VACCINIA VIRUS R.C. Condit, R. Pacha, Z. Fathi, U. Hooda, C. Thompson, E. Niles, P. Caro, and J. Soto, Department of Biochemistry, State University of New York at Buffalo, Buffalo, N.Y., 14214, USA

A summary will be presented of the genetics of vaccinia virus, with an emphasis on recent developments in the analysis of mutants affecting RNA metabolism, and in efforts to isolate conditionally lethal mutants in specific genes using directed approaches. Specifically, 66 ts mutants of vaccinia strain WR have been isolated, sorted into 31 complementation groups, phenotyped, and assigned map positions on the vrial genome using marker rescue. Of special interest are mutants in three complentation groups, two of which encode subunits of the vrial RNA polymerase, and one of which affects the stability of rRNA and viral mRNA after infection. The biology of these mutants is being studied in detail.

As a step toward isolation of conditionally lethal mutations in specific vaccinia genes using in vitro mutagenesis, a method has been developed which permits highly efficient targeted insertion of mutations into the vaccinia genome without selection. Refinements of this technique and its application to directed genetics in vaccinia will be discussed.

S9.5

Vaccinia DNA Replication, P. Traktman, Departments of Cell Biology and Anatomy and Microbiology, Cornell University Medical College, New York, NY, USA.

This talk will be an attempt to present the "state of the art" understanding of Vaccinia DNA replication. The work of several laboratories concerning the features of the hairpin termini of the genome, and their role in replication and genome structure, will be discussed. Likewise, work by others concerning the apparent lack of an origin (as defined by an "autonomous replicating sequence")as a requirement for replication in vaccinia infected cells will be discussed. Work from our own lab and others regarding the proteins encoded by genes known to be essential for viral replication will be discussed in detail. Analysis of viral DNA complementation groups will be described, as well as biochemical analyses of enzymes with presumed roles in replication and the maintenance of genome topology. Finally, the current status of in vitro replication systems for vaccinia will be reported.

S9.6

TRANSCRIPTION OF THE POXVIRUS GENOME

R. Wittek, C. Bertholet, E. Van Meir
Institut de Biologie animale, University of Lausanne, CH-1015 Lausanne, Switzerland.

The large poxvirus DNA contains about 200 genes, half of which are expressed prior to DNA replication and half of which are expressed late in infection.

Early transcription occurs within virus cores before the complete uncoating of the DNA. Exclusively viral enzymes are used for synthesis and modification of early mRNAs. The DNA sequences involved in the regulation of early gene expression are contained within only 30 nucleotides of 5' flanking sequence but these elements have not yet been characterized.

Expression of late genes depends on protein synthesis and DNA replication. Late mRNAs are about twice as long as early mRNAs and are very heterogeneous in size. In most late genes, the translation initiation codon is immediately downstream of the sequence TAA. This element, TAAATG, is necessary, but not sufficient for late gene expression.

Recent work from our laboratory revealed a very unusual structure at the 5' end of late mRNAs. In such molecules, the protein-coding sequences are preceded by long RNAs of viral or cellular origin, which are fused via a poly(A) stretch to the late gene ATG. This demonstrates that vaccinia virus produces late mRNA by a discontinuous mechanism.

S10.1

REGULATORY EVENTS IN PARAMYXOVIRUS REPLICATION. D. W. Kingsbury, D. S. Gill, E. M. Morgan, G.G. Re, K.W. Ryan, S. Takai. Department of Virology and Molecular Biology, St. Jude Children's Research Hospital, Memphis, Tennessee, USA.

We will describe strategies to define molecular mechanisms in the regulation of RNA synthesis by this family of nonsegmented negative-stranded RNA viruses. Recombinant DNA technology is being applied to Sendai virus to analyse structure-function relationships of the viral nucleocapsid proteins. Complete cDNA clones of the NP, P, and C genes have been inserted into vectors for expression in eukaryotic cells, and the assembly of a full-length L gene clone is in progress.

Also under study are functions of the cis-acting nucleotide sequences in the 50-base leader templates at each end of the genome, where positive- and negative-strand RNA syntheses are initiated, and in the 24-base consensus sequences at gene boundaries, where transcriptional control is exerted. We are developing a system for in vitro production of small viral RNA species (artificial deletion mutants) containing the regulatory sequences. If the small RNAs are replicated and transcribed in cells infected by nondefective helper virus, we will be able to observe the consequences of site-specific mutagenesis within the regulatory sequences.

S10.2

RIBOSOMAL INITIATION AT ALTERNATE AUGs OF THE SENDAI VIRUS P/C mRNA. J. Curran, and D. Kolakofsky. Department of Microbiology, University of Geneva Medical School, Geneva, Switzerland.

The Sendai virus P/C gene contains overlapping open reading frames (ORF). The P ORF (565 aa) starts on the 5' proximal AUG; the C ORF (205 aa) starts on the second AUG. The C ORF is known to code for a nested pair of proteins (C and C') which are C-co-terminal and we have recently found that the P ORF also expresses a second protein, called X, which represents the last 95 aa of P. To understand how ribosomes initiate at these alternate AUGs, site directed mutagenesis was used to change the context of each AUG, the distance between the P and C AUGs was altered, and oligonucleotides and RNAse H were used to cut the mRNA at defined locations. The results of these experiments suggest that whereas ribosomes must scan from the cap group to initiate at the P and C' AUGs, ribosomes appear to be able to directly access the C and X AUGs.

S10.3

MOLECULAR STUDIES ON THE STRUCTURE AND FUNCTION OF THE FUSION RELATED EXTERNAL DOMAIN OF THE FUSION PROTEIN OF THE PARAMYXOVIRUS SV5. R.G. Paterson and R.A. Lamb. Department of Biochemistry, Molecular Biology and Cell Biology, Northwestern University, Evanston, IL USA 60201

The hydrophobic NH_2 terminus (FRED) of the F1 subunit of the SV5 fusion (F) protein is implicated in mediating cell fusion, but in the inactive Fo precursor the FRED is translocated across membranes. If the FRED is hydrophobic enough to interact with the target membrane in mediating cell fusion, then the question arises as to how this hydrophobic region passes across the rough endoplasmic reticulum membrane during co-translational translocation of the nascent Fo precursor. We have constructed a series of hybrid proteins to enable us to study the effect of position on the properties of the FRED. The experiments indicate that the FRED is close to the threshold of hydrophobicity required to function as a membrane anchor. The FRED is not sufficiently hydrophobic to halt translocation across the lipid bilayer when in an internal position in the polypeptide chain; however, when placed at the carboxy terminus of a reporter molecule (anchorless hemagglutinin) the FRED is able to function to anchor the hybrid HA-F protein in membranes. The implications for biological activity will be discussed.

S10.4

RSV AND PIV3 GENE EXPRESSION. P. Collins, R. Olmsted, P. Johnson, M. Spriggs, B. Murphy. NIAID, NIH, Bethesda, MD 20892, U.S.A.

RSV and PIV3 gene products were analyzed with cDNA and RNA sequencing, recombinant SV40 and vaccinia viruses (VV) and antipeptide sera. Sequence comparison of the RSV A and B antigenic subgroups revealed extensive sequence divergence in the extracellular domain of the G protein (44% identity) compared to eight other RSV proteins (76-96%). Nonetheless, a VV expressing subgroup A G protein induced immunity in cotton rats to both subgroups. The extracellular domain of G contained a circumscribed conserved region that might be important in cross-subgroup immunity and receptor-binding. Deletion, truncation and chimeric mutants of G and the PIV3 HN protein expressed by SV40 and VV recombinants were analyzed to identify domains important in transport, O-linked glycosylation and biological activity. The RSV 1A protein was shown to be a membrane protein that is present in aburdance at the cell surface and is processed by alternate intracellular pathways that can include glycosylation steps. Immunization of cotton rats with recombinant VV provided protective immunity in the cases of F and G but not N and 1A, despite the surface localization of 1A. Between the subgroups, noncoding gene and intergenic sequences were nonconserved except for transcriptive signals and the overlap region between the 22k and L genes.

S10.5

THE ROLE OF INDIVIDUAL RESPIRATORY SYNCYTIAL VIRUS PROTEINS IN INFECTION AND IMMUNITY. G. W. Wertz[1], E. J. Stott[2], and L. A. Ball[1]. [1]Department of Microbiology, University of Alabama at Birmingham, AL 35294 USA; [2]A.R.F.C. Institute for Animal Disease Research, Compton, Newbury, Berkshire RG16 ONN, UK

Human respiratory syncytial (RS) virus remains a major cause of serious lower respiratory tract infection. In order to understand the role of individual viral gene products in infection and in the immune response, we placed complete cDNA copies for 8 of the 10 RS virus genes in eukaryotic expression vectors. The expression of these genes from several recombinant vectors was examined in cell culture. The results showed that biologically active RS virus proteins were expressed. The ability of recombinant vaccinia virus vectors that expressed individual genes to elicit a protective immune response was analysed in two animal model systems. Proteins that elicit a protective humoral or cell-mediated immune response were identified. The G and F surface glycoproteins of RS virus stimulated a strong humoral antibody response, and the lungs of mice that were vaccinated with these vectors were free of detectable RS virus following intranasal challenge. Protection by the G protein was subtype-specific, whereas protection by F was not. Furthermore, the N and F proteins were shown to be targets for the cell-mediated immune response.

S10.6

GENETIC DEFECTS AND GENOME VARIABILITY OF MEASLES VIRUS (MV) IN CASES OF SUBACUTE SCLEROSING PANENCEPHALITIS (SSPE). M. Billeter, R. Cattaneo, A. Schmid, G. Rebmann, K. Baczko*, V. ter Meulen*, S. Udem+. Institut für Molekularbiologie I, Universität Zürich, CH-8093 Zürich; *Institut für Virologie, Universität Würzburg, D-8700 Würzburg; +Albert Einstein College of Medicine, Yeshiva University, New York, USA.

Defective MV genomes were studied either in brain autopsies of SSPE patients or in cell lines carrying replicating SSPE-derived MV. Each case showed different gene expression defects recognizable by Northern blotting, in vitro translation or viral antigen detection. A specialized strategy now permits efficient derivation of full length cDNA clones corresponding to the 5 major MV mRNAs species from polyadenylated RNA of SSPE cases. Up to 1 percent of nucleotides varied in sibling clones of the same case indicating either particularly error-prone MV replication or relaxed selective constraints or both. Common in all defective MVs replicating in brain was an enhanced attenuation of transcription at each gene junction, causing unbalanced low expression of genes concerned with viral envelope formation.

S11.1

DEFECTIVE HOST IMMUNE RESPONSES IN VIRAL INFECTIONS
J.G.P. Simmons, L.K. Borysiewicz, J. Hickling, P.D. Mason. Dept. Medicine, Royal Postgrad. Medical School, London, UK.

Inferential evidence suggests effector T cell immunity is required to limit virus infection in the normal human host, although assessing the relative role of different effector T cells is constrained by the necessity to use in vitro systems. Here, the focus is on cytotoxic cells (Tc) and human herpes virus infections.

During asymptomatic persistent infection Tc-precursor cells (Tc-P) are present in peripheral blood - in the case of cytomegalovirus (CMV) and Epstein-Barr virus (EBV) their predominant specificity is probably for non-structural viral proteins expressed at early times in the viral cycle. The frequency of Tc-P specific for CMV or EBV is considerably greater than that for varicella zoster virus (VZV) - which has a different (neuronal) site of persistence. Defective generation of herpes virus specific Tc in vitro may be associated with predisposition to infection and/or with prolonged active infection (as with the syndrome of "chronic active" EBV infection) - increased Tc-P frequency may follow recovery suggesting restimulation in vivo. The role of non-MHC restricted Tc and natural killer cells in the resolution of infection is less clear. These in vitro observations bear on the possible use of methods to enhance cytotoxic cell effector function (for instance with IL2) or frequency (with specific viral antigen) in vivo.

S11.2

MONONUCLEAR CELLS AS A VECTOR FOR HUMAN CYTOMEGALOVIRUS INFECTION. J.A. Nelson and J.W. Gnann. Department of Immunology, Research Institute of Scripps Clinic, La Jolla, California, USA.

We have previously demonstrated by in situ hybridization that clinically healthy HCMV seropositive individuals harbor virus in circulating mononuclear cells. The predominant cell types which hybridize to an immediate early (IE) probe are monocytes and T-cells. Generally, we find that approximately 0.01% of the PBMN population hybridize to the IE probe. Hybridization with a late gene probe was negative but a few individuals had some positive cells. We also examined individuals with AIDS for expression of HCMV in PBMN cells. In this immunosuppressed population approximately 5-fold more cells hybridized with the IE probe and expression of late gene products was consistently higher compared to asymptomatic HCMV seropositive HIV seronegative patients. The importance of identifying monocytes and T-cells as potential vectors of HCMV infection was reinforced by studies examining the source of viral infection in kidney transplant patients. In these studies HCMV was not detected in the kidney parenchyma but in infiltrating inflammatory cells raising questions about the source and pathogenesis of HCMV infection in this population. Thus once an individual is infected with HCMV, the virus can be detected in the mononuclear cells which potentially can act as a vector for virus dissemination.

S11.3

HERPESVIRUS INFECTIONS IN TRANSPLANT RECIPIENTS
J. D. Meyers. Program in Infectious Diseases, Fred Hutchinson Cancer Research Center, Seattle, WA, USA.

Herpesvirus infections are common and may be severe in transplant recipients. The epidemiology is similar in recipients of renal, cardiac, marrow or liver grafts. Herpes simplex virus (HSV) infection is least problematic since the advent of acyclovir. Although reactivation rates of 80% may be expected among seropositive patients, reactivation may be prevented and disease treated with acyclovir. Life-threatening HSV infection such as pneumonia or encephalitis is rare even in severely immunocompromised patients. Cytomegalovirus (CMV) infection generally occurs within 2-4 months after transplant. Prevention is complicated by the multiple sources of infection: Primary infection from blood products or the organ itself may be prevented by serological screening of donors. Infection from latent virus in donor or host tissues must be prevented by prophylaxis with antiviral agents. Although still investigational, ganciclovir and foscarnet both show promise for the treatment of active CMV infection. Treatment of varicella-zoster virus (VZV) infection is possible with acyclovir, vidarabine or interferon. Acyclovir is preferred. The long-term use of acyclovir and the live VZV vaccine are potential approaches to prophylaxis. Finally, Epstein-Barr virus (EBV) infection plays a small, but important role in some patients. EBV-associated lymphoproliferative disease is the syndrome of most concern. Treatment and indeed the pathogenesis of this syndrome remain unclear.

S11.4

MECHANISMS OF HIV SUPPRESSION. M.S. Hirsch, Massachusetts General Hospital, Boston, MA, USA.

ABSTRACT NOT AVAILABLE

S11.5

RECOMBINANT HIV STRUCTURAL PROTEINS DETECT SPECIFIC CELLULAR IMMUNITY IN VITRO IN INFECTED INDIVIDUALS. T.C. Merigan[1], P.W. Berman[2], & J.W. Torseth[1]. Department of Medicine, Division of Infectious Diseases,[1] Stanford University School of Medicine, Stanford, CA, USA, & Department, Molecular Biology, Genentech,[2] Inc., South San Francisco, CA, USA.

Peripheral blood mononuclear cell (PBMC) cultures were established from patients with antibody to human immunodeficiency virus. Asymptomatically infected patients (5/19) had significant lymphocyte transformation responses induced in culture by a purified, recombinant envelope glycoprotein (rgp120) from the virus. A few (6/55) subjects with AIDS-related complex (ARC) and no subjects with AIDS (0/29) had proliferative responses to this protein. These responses correlated directly with circulating levels of helper/inducer lymphocytes ($p<0.01$) and in-directly with virus antigen in blood ($p=0.04$). Also, these responses occurred significantly less frequently than responses to herpes simplex virus (HSV) or cytomegalovirus (CMV) antigens in seropositive ARC patients ($p<0.005$). These data indicate that the frequency of immune cellular responses to rgp120 decline in association with disease progression, and become undetectable in frank AIDS. As rgp120 induced proliferation was not observed in cells from 13 seronegative immunocompetent subjects, this res-ponse appears immune specific. Immune T-lymphocyte-mediated responses to this HIV envelope glycoprotein may allow the prediction of future clinical events and may be useful in moni-toring immune-enhancing therapy in patients with ARC and AIDS.

S11.6

PROSPECTS FOR ANTIVIRAL CHEMOTHERAPY IN THE IMMUNO-COMPROMISED HOST. Bo Öberg. Department of Antiviral Chemotherapy, Research & Development Laboratories, Astra Alab AB, Södertälje, Sweden.

Chemotherapy against viral infections in the immunocompetent host often has a limited clinical benefit due to the rapid course of viral replication and the difficulty in administration of therapy early enough. This has been evident in viral diseases such as recurrent herpes, common cold and influenza.

Viral infections in the immunocompromised host are frequent and have a prolonged course. This long duration of viral replication makes effective chemotherapy a possibility. The most common viral diseases appearing as a consequence of the immunocompromised state are those caused by latent herpesviruses and some effective treatments of these diseases have appeared.

If the immunodeficiency remains severe, long-term treatment or prophylaxis may be necessary to control viral infections and this increases the risk for resistance development. A development of antiviral drugs with different modes of action is desirable both to manage resistance development and to find potential synergistic effects between different drugs.

S12.1

CRYPTIC PLANT VIRUSES: PROGRESS REPORT. G. Boccardo & G.P. Accotto. Istituto di Fitovirologia applicata, C.N.R., 10135 Torino, Italy.

To date, 30-38 nm isometric particles have been detected in a relatively large number of apparently healthy plant species belonging to at least six different botanical families. These particles sometimes occur in mixtures of different types. Where tested, cryptic viruses appear to be transmissible only through the progeny of carrier plants, the proportion of carriers varying depending on the status of the parents. Those cryptic viruses examined possess two or three segments of linear dsRNA. Cases have been reported where up to six dsRNAs could be isolated from purified virus particles. It now appears that mixtures of viruses were responsible for those findings. On the basis of morphology, a sub-grouping of these viruses has been proposed. The number of dsRNA segments found is not at the moment a reliable criterion for sub-grouping.

For diagnosis, various serological techniques and the electrophoresis of dsRNAs extracted from carrier plants have been employed. There are examples where dsRNAs extracted from carrier plants showed different patterns in gels than RNAs extracted from purified virus particles. Use of cloned cDNAs as diagnostic probes is for the moment very limited.

Where the genomic products have been identified in in vitro translation and immunoprecipitation experiments, these indicate that cryptic virus RNAs act as monocistronic genes, coding for the capsid protein and for another polypeptide of larger molecular weight, the functions of which remain to be established.

S12.2

MORPHOLOGY AND IN VIVO LOCATION OF CRYPTIC VIRUSES
R. G. Milne. Istituto di Fitovirologia Applicata del CNR, Via Vigliani 104, 10135 Torino, Italy.

Cryptic virus particles are isometric, falling into 2 size classes of diameter 29-32 nm (class A) and 37-38 nm (class B). "A" particles, e.g. those of white clover cryptic 1, beet cryptic 1 and 2, carnation cryptic 1 and vicia cryptic viruses are rounded, without distinct fine structure, and often penetrated by stain. "B" particles, e.g. those of white clover cryptic 2 or hop trefoil cryptic 2, have more prominent subunits. The two morphologies suggest that the cryptic virus 'group' can be divided into sub-groups A and B.

Cryptic virus particles have been found in phloem (ryegrass cryptic, radish yellow edge) and parenchyma (hop trefoil cryptic, white clover cryptic 1 and 2, vicia cryptic); it seems likely that the particles occur in most tissues. They have been found in both cytoplasm and nuclei. Radish yellow edge is the only cryptic virus for which viroplasms are reported. Otherwise there are no detected cytopathological effects. With white clover cryptic 1 and 2, sectored plants can arise in which some parts become virus-free. Sectoring is discussed in relation to the failure of graft transmission, and to transmission by pollen and ovule.

S12.3

RED PEPPER CRYPTIC VIRUS. T. Natsuaki, W. Wang, S. Okuda, M. Teranaka. Faculty of Agriculture, Utsunomiya University, Minemachi, Utsunomiya, 321 Japan.

Two cryptic viruses were found in red pepper (Capsicum annuum) and named red pepper cryptic virus 1 (RPCV 1) and RPCV 2 respectively. These viruses had properties similar to those of other cryptic viruses, such as (1) with isometric particles c. 30 nm in diameter, (2) high rates of seed-transmission, (3) low concentration in plants, (4) lack of symptoms in red pepper plants, (5) not transmitted by mechanical inoculation, and (6) containing double-stranded (ds) RNA segments with estimated mol. wt. of 1.14 and 0.92 x 10^6 for RPCV 1 and 1.06 and 0.98 x 10^6 for RPCV 2. In dot-blot hybridization with using photobiotin-labelled dsRNA segments of RPCV 1 & 2 as probes there was no cross-hybridization among them.

No cryptic virus was detected in sweet pepper (C. annuum var. grossum), eggplant and tomato. This is the first report of isolating cryptic viruses from solanaceous plants.

S12.4

SEROLOGY OF CRYPTIC PLANT VIRUSES. E. Luisoni. Istituto di Fitovirologia applicata, C.N.R., 10135 Torino, Italy.

More than 30 cryptic plant viruses have up to now been found, sometimes as mixtures. One or more antisera exist against 9 single viruses and 5 mixtures. Cryptic viruses are strongly immunogenic but are present in low or very low concentration in host tissues; therefore IEM and ELISA have been used for diagnosis in crude sap. IEM, and immunodiffusion with purified preparations, have also been used to detect serological relationships. Positive reactions have only been obtained within a group of three "large" and another of six "small" cryptic viruses of 5 leguminous species. Serologically different isolates of carnation cryptic virus 1 have also been detected.

As cryptic viruses are non-transmissible and give no symptoms, serology is fundamental for checking the carrier or non-carrier status of plants or parts of plants (with ELISA and IEM), and for distinguishing among different viruses of the same morphology often present in one plant (with IEM, using in particular heterologous sera).

Immunoprecipitation has been used both to obtain a monospecific serum from a polyspecific one and to separate one component from a virus mixture.

S12.5

ENDOGENOUS PLANT DOUBLE-STRANDED RNAs: CRYPTIC VIRUSES AND ORGANELLE-SPECIFIC AUTONOMOUSLY REPLICATING RNA'S. Ramon L. Jordan, USDA-ARS, BARC-W, FNCL, Beltsville, Md. 20705 USA.

Homogeneous high molecular weight double-stranded RNA (dsRNA) is not normally found in most cultivars of healthy plants. However, certain cultivars of presumably healthy plants have been shown to contain relatively large amounts of dsRNA. Some of these dsRNAs are similar in size and number (dsRNA pattern) to those normally associated with cryptic viruses (two or more major dsRNA species of 1.25 to 2.5 kbp). DsRNAs with distinct patterns not similar to cryptic virus dsRNAs have been isolated from at least one cultivar in each of 10 different plant families to date. These families (and number of distinct dsRNA patterns) include: Brassicaceae (2), Caryophyllaceae (4), Chenopodiaceae (3), Cucurbitaceae (5), Fabaceae (4), Lamiaceae (2), Lauraceae (2), Poaceae (3), Rosaceae (2), Solanaceae (4). These dsRNA patterns consisted of 1 to 3 dsRNA species with sizes ranging from 0.9 to 15 kbp. A few of these unique dsRNAs have been studied further. The 13-15 kbp dsRNAs isolated from 'Black Turtle Soup' bean (Phaseolus vulgaris) and 'California Wonder' pepper (Capsicum annuum) were shown to have sequence homology with their respective host plant chloroplast genome. Two dsRNAs (2.8 and 0.9 kbp) unique to S-type cytoplasm corn Zea mays have been shown to replicate in a DNA-independent manner and have sequence homology with mitochondrial ssRNA. A possible regulatory role via inhibition of gene expression for these endogenous dsRNAs has been suggested.

S12.6

MOLECULAR CLONING OF BEET CRYPTIC VIRUS DS RNA. J.F.Antoniw Plant Pathology Department, Rothamsted Experimental Station, Harpenden, Herts AL5 2JQ, U.K.

Beet cryptic virus (BCV) particles were purified from sugar beet cultivar Sharpes Kleine E and the four dsRNA components extracted. The dsRNAs were polyadenylated, denatured to produce ssRNA and then copied into cDNA and cloned into pUC9. Clones corresponding to three of the dsRNA components (1, 3 & 4) were identified. These did not hybridize to each other or to dsRNA2 suggesting that there is no significant sequence homology between the four dsRNA components.

RNA was extracted from the leaves of twenty beat plants and was analysed by Northern blotting using the cDNA clones as probes. In five plants the cDNA probes did not detect any BCV RNAs. But nine plants were found to contain BCV dsRNAs 1, 3 & 4 whereas in six plants only BCV ds RNAs 3 & 4 were detected. These results are compatible with the occurrence of two different beet cryptic viruses - BCV1 consisting of dsRNAs 1 & 2 and BCV2 consisting of dsRNAs 3 & 4. An antiserum prepared to purified BCV (a mixture of BCVs 1 & 2) was used in immunosorbent electron microscopy to assay for BCV in the same twenty plants. The antiserum was more sensitive for the detection of BCV1 than BCV2, but was not as sensitive or as specific as the cDNA probes in hybridisation assays. Some sequences of the cDNA clones will be presented.

S13.1

THE ENTRY AND ASSEMBLY STRATEGY OF ENVELOPED DOUBLE STRANDED RNA PHAGE Ø6. D.H.Bamford, M.Romantschuk, V.M.Olkkonen. Department of genetics, University of Helsinki, Helsinki, Finland.

Bacteriophage Ø6 is an enveloped RNA virus of Pseudomonas syringae. The genome consists of three segments of dsRNA. The phage reseptor is a chromosomally coded pilus. Pilus retraction brings the virion to the cell surface where the phage envelope fuses with the bacterial outer membrane with a mechanism which is dependent of a phage membrane protein but divalent cation and pH independent. After fusion a virion associated lytic enzyme locally digests the peptidoglycan, allowing the nucleocapsid (NC) to pass through the periplasmic space. During the NC penetration the NC surface protein is left behind and RNA and at least three NC proteins enter the cell through the cytoplasma membrane (CM). These proteins are components of the phage RNA polymerase. The CM penetration step is dependent on the proton motive force.

During assembly procapsids can be formed in the presence of two of the NC proteins. The procapsid matures by packaging of the RNA segments and by addition of the rest of the NC proteins. The NC is wrapped in an envelope containing phage specific proteins only. This takes place in the cytoplasm but obviously in contact with the CM. Mature phages are released by disruption of the host cell.

S13.2

DNA PACKAGING BY BACTERIOPHAGE P22. S. Casjens, R. Parr and S. Randall, Department of Cellular, Viral and Molecular Biology, University of Utah Medical Cenier, Salt Lake City, Utah, U.S.A.

Only five phage gene products are required to assemble P22 virion shells and package the dsDNA chromosome. Three of these, the products of genes *1, 8* and *5*, are structural components of the precursor particle (the prohead) and two, the products of genes *2* and *3*, are required transiently during DNA packaging. The latter two proteins are thought to be involved in the recognition of the substrate DNA for the packaging apparatus. Phage DNA is replicated as a concatemer and a single chromosome length molecule is cleaved from this oversized substrate during the packaging reaction.

We have analyzed three types of missense mutations which affect the *in vivo* function of the gene *3* protein, and these strongly support the idea that the phage DNA concatemer is recognized primarily by the gene *3* protein. One of these mutations alters a single amino acid in the *3* protein and causes a dramatic change in the target sequence recognized by that protein. These mutants have also allowed us to tentatively identify the recognition or *pac* site on the DNA that is presumed to come into contact with the *3* protein during initiation of DNA packaging. Other missense mutations implicate the gene *1* protein in the process of DNA entry into the prohead.

S13.3

REGULATION OF LAMBDA SITE-SPECIFIC RECOMBINATION BY HOST PROTEINS. J.F. Thompson, L. Moitoso de Vargas, C. Koch, R. Kahmann and A. Landy. Division of Biology and Medicine, Brown University, Providence, RI USA, and Max Planck Institut fur Molekulare Genetik, Otto Warburg Laboratorium, Berlin, West Germany.

Bacteriophage lambda has evolved a complex regulatory system to couple its development with environmental conditions. Because lambda is necessarily capable of monitoring only a limited number of cellular characteristics, the proteins that it has selected to participate directly in the recombination reactions are likely to play an important role in the global regulation of the cell. The two proteins involved, IHF and FIS have pleiotropic effects on gene expression and differentially regulate the direction of recombination. The mechanism by which these proteins can stimulate and inhibit the recombination reactions will be discussed. These regulatory effects will be placed in the context of the in vivo function of the proteins.

S13.4

ORGANIZATION OF THE GENOME OF THE SHIGA-LIKE TOXIN (SLT) CONVERTING BACTERIOPHAGE H-19B. J. Brunton, A. Huang, S. De Grandis, J. Ginsberg and J. Friesen. Departments of Microbiology, Medicine and Medical Genetics, University of Toronto and the Mount Sinai Research Institute, Toronto, Ontario.

Bacteriophage H-19B has a 48 Kb double stranded DNA genome which was shown to possess cohesive termini. The putative phage attachment site was mapped and the relationship of H-19B to lambda was examined by Southern hybridization. Nick translated H-19B DNA hybridized to three restriction fragments of lambda which carry the J gene, the int/xis and the O and P genes respectively. In reciprocal hybridizations, three homologous areas were identified in the H-19B chromosome which were organized in a fashion similar to the organization of the homologous regions of lambda. The SLT cistrons were located between the area homologous to the O and P genes and the right cohesive terminus in an area analogous to the ninR region of lambda. Studies of SLT transcripts and the DNA sequence showed that the two toxin cistrons are organized as an operon. Transcription is in the same direction as the putative rightward operon of H-19B. A sequence characteristic of rho independent terminators is located 270 BP downstream from the termination codon of the B subunit cistron. We conclude that H-19B could be classified as a lambdoid bacteriophage. The SLT operon is located far from the phage att site suggesting that it is unlikely that it was picked up by imprecise excision of a prophage.

S13.5

PROTEINS INVOLVED IN THE TRANSPOSITION OF BACTERIOPHAGE MU DNA. G. Chaconas, S.J. Buch, G. Gloor, and M.G. Surette. Department of Biochemistry, University of Western Ontario, London, Ontario, Canada, N6A 5C1.

Transposition of bacteriophage Mu DNA is an obligatory step in the lifecycle of the phage; the transposition frequency of Mu DNA is therefore orders of magnitude higher than that of other movable elements. Infecting Mu DNA is injected into the cell along with the 64 kDa virion N protein. This protein circularizes the infecting DNA by interacting non-covalently with the ends of the DNA. The supercoiled protein-DNA complex is a substrate for transposition catalyzed by the Mu early proteins A and B in conjunction with host proteins. Early during transposition, stable protein-DNA complexes are involved in the process. These stable complexes, or transpososomes, can be generated in vitro and correspond to pre-synaptic and synaptic complexes. The characterization of these transpososomes and their role in DNA transposition will be discussed.

S13.6

REPLICATION OF PHAGE Ø29 DNA PRIMED BY THE TERMINAL PROTEIN. M. Salas, A. Zaballos, C. Escarmis, C. Garmendia, J. Gutiérrez, I. Prieto, G. Martín, A. Bernad, L. Blanco, J.M. Lázaro, D. Guirao, M. Serrano, M.A. Blasco, M.J. Otero, R.P. Mellado, J.M. Hermoso, Centro de Biología Molecular, Universidad Autónoma, Canto Blanco, 28049 Madrid. Spain.

Phage Ø29 has a linear DNA with protein p3 linked to each 5' end by a phosphodiester bond between serine residue 232 and dAMP. Replication starts at either end of the DNA-p3 template in the presence of terminal protein p3 and Ø29 DNA polymerase p2, by the formation of a p3-dAMP complex that is elongated to produce full-length Ø29 DNA. Deletion mutants at the amino and carboxyl ends of protein p3, as well as site-directed mutants, have been constructed and their activity in the formation of the p3-dAMP complex determined. The viral proteins p5 and p6 have been purified. Protein p5 binds to single-stranded DNA. Protein p6 stimulates the in vitro initiation and elongation reactions and binds to double-stranded DNA. The template requirements in Ø29 DNA replication have been studied by in vivo transfection experiments using recombinant Ø29 DNA and by site-directed mutagenesis of the cloned replication origins used as templates in vitro.

S13.7

THE LIFE CYCLE OF BACTERIOPHAGE ØX174: THE SEQUENCES REQUIRED FOR INITIATION AND TERMINATION OF DNA REPLICATION. A.D.M. van MANSFELD, A.C. Fluit, P.D. Baas and H.S. Jansz. Inst. Mol. Biol. and Lab. Physiol. Chem., Univ. Utrecht, Utrecht, NL

Gene A protein of bacteriophage ØX174 is a site-specific endonuclease which initiates ØX rolling circle DNA replication by cleaving the viral strand of double-stranded replicative form DNA at a unique site, the origin. Cleavage creates a free 3'-OH primer for DNA synthesis at one end and a covalent gene A protein-DNA complex at the other (5')end of the cleavage site. Termination of a replication round, which involves cleavage of the rolling circle at the regenerated origin and circularization of the genome-length viral DNA, is accomplished by the covalently linked gene A protein. The cleavage site is located in a region of 30 nucleotides (origin region) whose sequence has been highly conserved among ØX and other isometric single-stranded DNA phages. Following different approaches we have shown that gene A protein has different DNA-sequence requirements for the different reactions which are catalyzed. A model for the interaction of gene A protein with the different domains in the origin region which is based on these findings will be presented. Analysis of the covalent gene A protein-DNA complex has shown that 2 different tyrosyl residues in gene A protein can bind to the DNA. The two tyrosyl residues lie only 3 amino acid residues apart. Based on this finding we propose a mechanism by which gene A protein can cleave, and cleave and ligate DNA during initiation and termination of ØX DNA replication.

S14.1

MOLLICUTES AND THEIR VIRUSES. J.M. BOVE. Laboratoire de Biologie Cellulaire et Moléculaire, INRA et Université de Bordeaux II, Domaine de la Grande Ferrade, B.P. N° 131 - 33140 PONT DE LA MAYE, France.

Mollicutes are the smallest and simplest self replicating procaryotes, arising from low (G + C) Gram positive bacteria by degenerative evolution. A first genome reduction to 1000 MDa and loss of cell wall led to the Acholeplasmas seen as the first mollicutes. An early splitting of the Acholeplasma branch gave, without genome reduction, the sterol requiring organisms ancestral to *Spiroplasma* sp. Repeated independent genome reductions each to about 500 MDa led, during evolution of the Spiroplasma branch, to the *Mycoplasma* sp. and the *Ureaplasma* sp.. *Anaeroplasma* sp. and *Asteroleplasma* sp. are anaerobic mollicutes. The Anaeroplasma branch is probably close to the node between Acholeplasma and Spiroplasma branches.

Many mollicutes are pathogens of animals. Some produce human diseases. Among the 30 or so recognized helical mollicutes (spiroplasmas), three are plant pathogenic, one produces an experimental disease in suckling mice and others are insect pathogens. Mollicutes are also frequent contaminants of cell cultures.

Acholeplasmas can be infected by five different types of viruses, Mycoplasmas by 3 and Spiroplasmas by 4. The virions are naked rods, naked isometric spheres, enveloped irregular spheres, polyhedrons with short tails or polyhedrons with long tails. Spiroplasma virus SpV4, a naked isometric particle with single stranded circular DNA, has been used as a model to study molecular features of the spiroplasmas. From the nucleotide sequence of its genome, information on open reading frames, promoters and terminators, ribosomes buding sites and codon usage could be deduced. It has also been found that in Spiroplasmas as in *M. capricolum* (YAMAO et al., P.N.A.S., 82, 1985, 2306), UGA is not a termination codon, but codes for tryptophane.

S14.2

MYCOPLASMA INFECTION OF CELL CULTURES: INCIDENCE AND CHARACTERISTICS. J. A. Robertson. Department of Medical Microbiology and Infectious Diseases, Faculty of Medicine, University of Alberta, Edmonton, Canada.

The contamination of cell cultures by mycoplasmas is widespread. The mycoplasmas are more likely to be found in continuous cell lines than in primary cell cultures. At least 15% of cell lines and virus stocks carry these small prokaryotes. However, because of their small cell mass, little or no turbidity is produced and the contamination may not be noticed. The consequences of mycoplasma contamination are diverse; the outcome of a particular infection is largely dependent upon the species of mycoplasma and the type of cell culture. The introduction of mycoplasmal macromolecules and enzymatic activities frequently alters eukaryotic cell structure and function. Unusual metabolic patterns, cytopathic effects and permanent chromosomal aberrations are among the observed effects. In mycoplasma-contaminated cultures, the expression of viral proteins and the yields of virus may be altered. In addition, mycoplasmas induce interferon production. Whether the effect of the contaminating mycoplasma is minor or profound, its unintentional presence in cell cultures invalidates experimental data, leading to confusion instead of enlightenment.

S14.3

METHODS TO DETECT AND PREVENT MYCOPLASMA INFECTION. G. J. McGarrity and H. Kotani, Coriell Institute for Medical Research, Camden NJ USA 08103

The incidence of mycoplasmal infection (MI) of cell cultures is influenced by quality control programs, as well as the frequency and efficiency of detection methods. Microbiological assays will not detect all cell culture mycoplasmas. Additional procedures are required. The use of DNA fluorochromes has been highly efficient to detect MI. This should be done in an indicator cell culture rather than the unknown specimen to allow improved standardization and control. The efficiency of this assay is 98-99%, based on tens of thousands of assays. The use of 6 methylpurine deoxyriboside (6MPDR) has also been effective in limited studies. 6MPDR has no discernible effect on mycoplasma free cell cultures. In the presence of mycoplasmas, 6MPDR is enzymatically cleaved to two anti-metabolites which kill cells in 3-4 days. Use of mycoplasma specific genetic probes has been introduced and offers potential to detect and identify mycoplasmas.

Prudent, simple and effective measures are available to prevent introduction of mycoplasmas into cell cultures. These will be listed, as will some methods to eliminate mycoplasmas from infected cell cultures when culture replacement is not possible.

S14.4

THE MYCOPLASMA ARTHRITIDIS T CELL MITOGEN: A NEW MODEL SYSTEM FOR THE STUDY OF THE GENETICS AND MECHANISMS OF T LYMPHOCYTE ACTIVATION. B. C. Cole, Department of Internal Medicine, Faculty of Medicine, University of Utah, Salt Lake City UT, USA

Mycoplasma arthritidis, a natural arthritogenic agent for rodents, produces a soluble, basic, low M_r (15kD) protein which is mitogenic for mouse, rat and human T lymphocytes. The mitogen, MAM, is active at $\leq 10^{-11}$ M and can associate with large M_r serum and medium components.

MAM has unique properties. Activation (proliferation, cytolytic action; γ interferon and IL-2 production) of T lymphocytes or T hybridomas requires the presence of accessory cells (AC) expressing the E_α chain of the MHC coded I-E molecule which acts as the receptor for MAM; AC processing of MAM is not required. Unlike antigen-induced T cell activation, T cells need not recognize self MHC products as long as MAM is presented in association with E_α. The T cell receptor for MAM-Eα is distinct from the antigen-receptor. MAM is also clonally restricted and its high potency is due in part to recruitment of other T subsets.

Non MHC genes have been identified which confer an inability of Eα bearing splenocytes to respond to MAM. This lack of response lies with the T cell rather than the AC and is specific for MAM since T cells respond normally to plant lectin mitogens. The combined data suggest that MAM activates T cells via a hitherto unknown pathway.

S15.1

ANTIGEN AND EFFECTOR T CELLS IN INFLUENZA INFECTION. Andrew J. McMichael, Nuffield Department of Medicine, John Radcliffe Hospital, Oxford, UK

Activation of cytotoxic T lymphocytes (CTL) is a normal part of influenza virus infection. Insure blood donors have circulating cytotoxic T memory cells which can be stimulated in vitro by influenza A virus. The level of these circulating memor cells is varied in different individuals and changes with time. They can be restimulated by influenza A virus infection. Studies in mice indicate that they are involved in recovery from influenza infections and we have shown a correlation between levels of cytotoxic T cell activity and recovery from infection in studies in volunteers.

Human influenza A virus specific CTL show specificity for influenza virus type, A or B, and HLA Class I antigens on the surface of infected cells. Using recombinant vaccinia viruses to insert single influenza virus genes into target cells we have found that human CTL recognised predominantly the nucleoprotein, matrix protein and polymerase PB2. Because these proteins are conserved, this accounts for the cross-reactivity of CTL for influenza A viruses of different subtypes. HLA type played a role in determining which virus antigen was recognised. Further studies have indicated that CTL recognise short synthetic peptides derived from the sequences of NP and M proteins. One peptide, nucleoprotein 335-349, was seen by all individuals of the HLA type B37 and no others. A second peptide, matrix 57-68, was recognised by CTL from all individuals from with HLA-A2. CTL from individuals with variant HLA-A2 molecules did not recognise this peptide and studies with mutant HLA-A2 molecules, made in vitro indicate that single amino acid changes in the A2 molecule effect recognition of this peptide.

The results suggest a very precise role for HLA Class I molecules in presenting influenza virus antigens to cytotoxic T lymphocytes. Given the role of CTL in the recovery phase of influenza infection, the results suggest that variability in response to influenza A virus infection may in part be influenced by HLA type.

S15.2

REGULATION OF H-2 CLASS I EXPRESSION IN VIRALLY INFECTED OR TRANSFORMED CELLS: IMPACT ON RESISTANCE TO TUMORIGENESIS. Daniel Meruelo. Department of Pathology and Kaplan Cancer Center, New York University Medical Center, New York, New York 10016.

Our group first documented that viral infections and virus-induced transformations can significantly affect class I gene expression. Simultaneously we demonstrated the impact of altered H-2 antigen expression on the effectiveness of the host's immune response to virus-infected cells. Subsequently, investigators using several other systems including RNA (AKR, MoLV, VSV) and DNA (HSV, SV40, Polyoma, Adenovirus) tumor viruses have reported similar alterations in class I gene expression. In RadLV, Ad, HSV, VSV, and MoLV systems, a minimum critical level of surface H-2 expression is required for CTL recognition.

In the RadLV system H-2D antigen expression is elevated immediately after virus inoculation on thymocytes of resistant but not of susceptible mice. After RadLV infection, viral antigen expression is greater in susceptible animals which show little if any changes in H-2 antigen expression. In virus-infected resistant mice, which display maximal H-2 antigen expression, rapid elimination of viral antigen expressing cells occurs. Finally, H-2 antigens usually disappear from the surface of RadLV transformed cells when overt leukemia develops. The molecular and cellular basis for these observations will be discussed.

S15.3

VIRUS-INDUCED CELL-MEDIATED AUTOIMMUNE REACTIONS TO BRAIN ANTIGENS. V. ter Meulen, P.T. Massa, U.G. Liebert, R. Dörries and H. Wege. Institut für Virologie und Immunbiologie, Universität Würzburg, Versbacher Str. 7, D - 8700 Würzburg, F.R.G.

Cellular changes occurring during replication and assembly of many viruses have long been thought to lead to autoimmune reactions in infected hosts and consequently to subacute or chronic diseases. We have studied this aspect in two animal models utilizing Lewis or BN rats infected with either murine coronavirus JHM or measles virus. In both models, rats develop a subacute inflammatory CNS disease process on the basis of a persistent infection which is accompanied by a cell-mediated immune response to myelin basic protein in Lewis rats, but not in BN rats. This immunogenetically controlled autoimmune reaction can be correlated with the ability of these viruses to induce extremely high levels of Ia molecules on susceptible rat brain astrocytes. It could further be shown that specific epitopes of structural proteins of these two viruses are responsible for the Ia induction. These findings suggest that the immunomodulatory capacity of these two agents may play a role in the induction of the observed autoimmune reaction.

S15.4

T-CELLS IN INFLUENZA AND RESPIRATORY SYNCYTIAL VIRUS (RSV) INFECTION. B.A. Askonas, Division of Immunology, National Institute for Medical Research, London, England.

T-cells play an important role in clearing virus in many infections but in some instances also lead to enhanced immunopathology. We therefore need to understand the recognition patterns and function of T-cell subpopulations in different infections.

In this introduction to the session, I shall discuss the effect of cloned cytotoxic T-cells (Tc) and T-helper cells (T_H) in virus replication and lung histology in a mouse model of influenza infection. Tc cells are more effective in clearing influenza from lung and trachea than T_H cells while RSV specific Tc exacerbate disease in RSV infection.

S16.1

AUTONOMOUS GENETIC ELEMENTS HEREDITARILY MAINTAINED IN TRANSGENIC MICE AND THEIR POSSIBLE USE AS GENE VECTORS. Minoo Rassoulzadegan, Pierre Léopold, Joëlle Vailly and François Cuzin, Unité INSERM 273, Centre de Biochimie, Université de Nice, 06034 Nice, France.

Two unexpected observations were made on transgenic mouse strains established by microinjecting into fertilized eggs a mixture of two plasmid DNAs. One of them, pPyLT1, encodes the large T antigen of polyoma virus. The other plasmid, termed pCG4, was initially present as a very minor contaminant present in the original DNA solution, but is necessary for the observed effect. It had been isolated in our laboratory by selecting cellular mouse sequences to which polyoma large T antigen was bound in transformed mouse cell lines. Upon injection of a 1,000:1 mixture of pPyLT1 DNA, transgenic animals are produced with very high frequencies (90 to 100% of the progeny from the microinjected eggs), and at least part of these animals maintain autonomous genetic elements which are efficiently transmitted in a non-mendelian manner. Functional bacterial vector sequences can be maintained, and the plasmids, recovered in Escherichia coli by transfection.

S16.2

DEVELOPMENT OF SINDBIS VIRUS AND DEFECTIVE INTERFERING PARTICLES AS EXPRESSION VECTORS. S. Schlesinger, R. Levis, C. Xiong, C. Rice, H. Huang. Department of Microbiology and Immunology, Washington University School of Medicine, St. Louis, MO 63110

We are exploring the possibility of using the positive strand RNA virus, Sindbis virus, and its defective interfering (DI) particles as vectors for introducing foreign genes into cells. In previous work we showed by deletion mapping of a cloned cDNA derived from one of the DI RNAs that only nucleotides at the 3' and 5' termini of the RNA are essential for the DI RNA to be amplified after it is transfected into cells in the presence of helper virus. As a first step in developing a vector we replaced 75% of the internal nucleotides of this DI cDNA with foreign sequences including the bacterial chloramphenicol acetyltransferase (CAT) gene. DI RNAs transcribed from this cDNA were replicated and packaged by helper Sindbis virus and became a major viral RNA species in infected cells by the third passage after transfection. They were also translated to produce enzymatically active CAT.

We have also inserted the CAT gene into the cDNA of the Sindbis virion genome in place of the structural protein genes. RNA transcribed from this cDNA, when transfected into cells, is self-replicating and produces active CAT. When this RNA is transfected in the presence of Sindbis virus it is packaged.

S16.3

RETROVIRUS VECTOR SYSTEMS, R. Mulligan, Whitehead Inst. for Biomedical Research, Cambridge, MA, USA

ABSTRACT NOT AVAILABLE

S16.4

BACULOVIRUS FOREIGN GENE EXPRESSION VECTORS. Part of Symposium S16. Max D. Summers, Texas A&M University and Texas Agricultural Experiment Station, College Station, Texas 77843..U.S.A.

The baculovirus vector (BV) utilizes the Autographa californica nuclear polyhedrosis virus (AcNPV) polyhedrin promoter modified for the insertion of foreign genes. This allows expression of procaryotic or eucaryotic genes to produce fused or non-fused recombinant proteins. One of the major advantages for use of this invertebrate virus expression vector over bacterial, yeast and mammalian expression systems is the very abundant expression of recombinant proteins from foreign genes linked to the polyhedrin promoter. Recombinant proteins are secreted; targeted to the nucleus; N-linked glycosylated; antigenically, immunogenically and functionally similar to their authentic counterparts. The BV is not pathogenic to vertebrates or plants and does not employ transformed cells as do the mammalian expression systems. The BV also provides the convenience of many of the protein processing systems which occur in the higher eucaryotic cells and which may be essential for the complete biological function of a recombinant protein.

S16.5

VACCINIA VIRUS GENE VECTORS. J.P. Lecocq, M.P. Kieny, G. Rautmann, B. Guy, D. Spehner, R. Drillien. Transgene S.A., 11 rue de Molsheim, 67000 Strasbourg, France.

Vaccinia virus was extensively used for immunoprophylaxis of small pox and enabled the eradication of the disease in 1977. Fundamental studies of vaccinia virus have nevertheless continued and recently, genetic engineering technology has permitted its development as a cloning and expression vector.

Recombinant vaccinia viruses immediately appeared as a very attractive way to elaborate new live vaccines. This concept rapidly progressed in the last years and genes encoding numerous antigens have been integrated into the vaccinia virus genome. Results obtained with the rabies glycoprotein are probably the most spectacular: foxes and racoons, which constitute the natural reserve of rabies virus, have been shown to be protected following a single oral administration of the recombinant vaccinia virus.

The high efficiency of the system led immediately to envisage it as an attractive vaccine candidate against AIDS. Recombinant vaccinia viruses expressing various HIV1 antigens have been constructed and the most recent results will be presented and discussed.

The concept of recombinant viruses as live vaccines has recently been extended to other poxviruses; results obtained namely with fowlpox offer new perspectives in the veterinary field.

S16.6

HERPES SIMPLEX VIRUS GENE VECTORS. M. Arsenakis, Dept. of Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL, USA

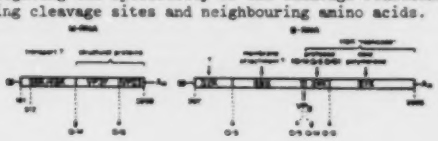
ABSTRACT NOT AVAILABLE

S17.1

ANALYSES OF THE FUNCTION OF THE COWPEA MOSAIC VIRUS ENCODED 24K PROTEASE BY TRANSLATING IN VITRO SYNTHESIZED MUTANT RNAS
J. Wellink, P. Vos, M. Jaegle, J. Verver, R. Goldbach and A. van Kammen. Dept. of Molecular Biology, Wageningen Agricultural University, The Netherlands.

The genome of cowpea mosaic virus (CPMV) consists of two messenger-sense RNAs designated M- and B-RNA. Both RNAs are translated in vitro and in vivo into large polyproteins which are subsequently processed by proteolytic cleavages into smaller functional proteins. Processing occurs at three types of cleavage sites: gln-met(Q/M),gln-gly(Q/G)and gln-ser(Q/S).

By translating in vitro transcripts of modified cDNA clones of B-and M-RNA, the 24K protein was shown to be able to cleave the Q/M,Q/S and Q/G sites in the B-RNA-encoded polyprotein and the Q/G site in the M-RNA-encoded polyprotein. In contrast to earlier experiments no proteolytic activity appeared to be associated with the 32K protein. However the Q/M site in the M-RNA-encoded polyprotein was found to be cleaved only in the presence of both intact 24K and 32K protein. Currently we are investigating the specificity of the cleavage reactions by changing cleavage sites and neighbouring amino acids.



S17.2

GENOME STRATEGY OF ALPHAVIRUSES AND FLAVIVIRUSES.
E.G. Strauss, The Australian National University, Canberra, Australia

We have been studying the genome organization of alphaviruses and flaviviruses using a comparative approach, that is by obtaining the nucleotide sequence of a number of members of each group. Since within coding regions, codon usage has been randomized even in domains of almost total amino acid conservation, those regions in which nucleotide sequence and/or secondary structure is conserved are of special interest. Such elements probably function as recognition sites or promoters for replication/transcription and may also be employed for temporal control of particular events during replication. A number of such elements have now been identified in both alphaviruses and flaviviruses and are being investigated both by comparative sequencing and by direct manipulation of cDNA copies of the genomic RNAs.

Both alphaviruses and flaviviruses translate their genomes as polyprotein precursors whose processing is believed to require both virus encoded proteases and cellular organelle-bound proteases. Furthermore some alphaviruses, but not all, regulate synthesis of a nonstructural protein by low frequency readthrough of an opal termination codon. These processing steps are also being studied by comparison of related viruses, sequencing of temperature sensitive mutants, and by site-specific mutagenesis of cDNA clones.

Similarities in genome organization and amino acid sequence of virus encoded proteins suggest that all of the eukaryotic RNA viruses share a common ancestry. Sequencing studies have significant implications for viral taxonomy and should lead to a clearer understanding of viral evolution.

S17.3

PROCESSING OF PICORNAVIRAL POLYPROTEINS. Ann Palmenberg. Biophysics Laboratory, University of Wisconsin, Madison, Wisconsin, USA.

Picornaviral RNAs encode a single, long open reading frame representing most of the theoretical coding capacity of the genome. Translation produces a giant precursor protein, which is cleaved in an orderly series of proteolytic steps to yield mature viral peptides. The processing cascade requires at least three different viral-encoded proteolytic activities (2A/2B, 3C and VP0), each of which has unusual monomolecular and/or bimolecular mechanistic properties.

Maturation cleavage of capsid precursor peptide VP0, for example, probably occurs by a novel, autocatalytic serine-type reaction in which viral RNA serves as proton acceptor and may actually help trigger the proteolytic catalysis during virion morphogenesis.

Site-directed mutagenesis experiments with viral cDNA segments have been employed to study the substrate requirements of the 2A/B and 3C proteases as well as to examine potential cross-reactivity among proteases from different viral genera. For the 3C protease, the data suggest that specific amino acid sequence contexts in accessible, flexible, turn-coil secondary structures are required elements for cleavage site recognition.

S17.4

Proteins involved in flavivirus replication complexes. M. Brinton and J. Grun. The proteins of viral replication complexes must function to provide template recognition and initiation, elongation, capping, and release of nascent strands. Flavivirus genome RNA encodes 7 non-structural proteins whose functions have not been characterized. Viral polymerase and methyltransferase activities have been demonstrated in crude cytoplasmic extracts from West Nile virus (WNV)-infected BHK cells. Further purification of these activities by gradient centrifugation removed the largest non-structural protein and a number of cellular proteins, but had little effect on either activity. The loss of the NS5 protein was unexpected, since this protein contains a canonical Gly-Asp-Asp sequence conserved among RNA virus polymerase proteins. It is possible that the NS5 protein is part of a separate replication complex which copies minus strands from plus strand templates. Three small hydrophobic viral proteins, ns4a, ns2a, and ns2b, as well as the NS3 remain associated with the majority of the polymerase and methyltransferase activity. Monospecific polyclonal antibodies are being utilized to identify the protein(s) that function(s) to elongate and/or cap nascent RNA strands. Some cellular proteins co-purify with the viral replication complexes. The specific involvement of at least one cellular protein in flavivirus RNA synthesis is indicated by the existence of a murine flavivirus-resistance gene; cells from resistant animals synthesize WNV full-size genomic RNA inefficiently.

S17.5

Structure of Sindbis virus.

S. D. Fuller. European Molecular Biology Laboratory. Heidelberg Fed. Rep. Germany

The three dimensional structure of Sindbis virus and of isolated Sindbis virus nucleocapsids have been determined by cryoelectron microscopy and image reconstruction. The spikes of the virus form columnar trimers arrayed on a T=4 lattice. The lipid bilayer is polyhedral in intact virions. The structure of the nucleocapsid seen within the virion matches that seen after isolation and has T=3 icosahedral symmetry. The arrangement of spike proteins is complementary to that of the capsid. Two types of capsid-spike interactions are seen: tight ones for the sixty trimers near the five-fold ones and looser ones for the twenty on the three-fold ones. Sequence alignment with picornaviral vp3's in combination with the structural results suggests that the capsid protein has the same eight-strand β barrel fold as seen for other T=3 RNA viruses but is akin to their expanded forms.

S17.6

AUTOLYTIC PROCESSING OF SATELLITE TOBACCO RINGSPOT VIRUS RNA. George Bruening, Department of Plant Pathology, University of California, Davis, California 95616, U.S.A.

Small satellite RNAs of plant viruses exhibit biological dependence, replicating and becoming encapsidated only in mixed infections with a supporting virus. Almost no information about the replication of the 359 nucleotide residue (nt) satellite tobacco ringspot virus RNA (STobRV RNA) is available from in vivo experiments, but in vitro observations support a specific model. Infected tissues have repetitive sequence multimeric forms of STobRV RNA of both polarities, as well as circular forms. Dimeric STobRV RNA from virus-like particles and transcripts of each polarity, from dimeric cDNA clones, autolytically process. The processing sites for the (+), i.e., encapsidated, and for the (-) polarity RNAs are 49 nt apart in the STobRV RNA sequence and have distinct nt sequences. The terminal groups after processing are a 5'-OH and a 2':3'-cyclic phosphodiester. Thus autolytic processing is a phosphotransfer reaction rather than hydrolysis. Each reaction is reversible to give circular forms of the monomeric STobRV RNA. This non-enzymic ligation occurs considerably more readily for STobRV (-)RNA than for STobRV (+)RNA. The autolysis and ligation reactions that have been observed in vitro, together with transcription of circular templates, form a scheme for the replication of STobRV RNA in vivo.

S18.1
G. Miller, Dept. of Pediatrics, Yale University, New Haven, CT, USA

ABSTRACT NOT AVAILABLE

S18.2
EPSTEIN-BARR VIRUS GENE EXPRESSION IN NASOPHARYNGEAL TISSUE. J.R. Arrand, A.C. Goodeve and J.D. Tugwood. Department of Molecular Biology, Paterson Institute for Cancer Research, Manchester M20 9BX, England.

Epstein-Barr virus (EBV) is a ubiquitous Herpesvirus, latently infecting B lymphocytes of 90% of adult humans worldwide. EBV is implicated in the aetiology of Nasopharyngeal carcinoma (NPC). Nasopharyngeal biopsy tissue samples from both normal EBV seropositive individuals and those with NPC have been examined for the presence of EB viral DNA and its transcriptional products in an attempt to elucidate the virus' role in normal and malignant nasopharynx. NPC tissue has been demonstrated by in-situ hybridisation to contain significantly more EBV DNA than normal nasopharyngeal tissue. In normal nasopharynx, the EBV genome appears to be present in low copy number in occasional cells. EBV transcription in nasopharyngeal tissue has been studied by Southern blotting and probing with in-vitro labelled cytoplasmic RNA. Normal nasopharyngeal tissue demonstrates a "latent" pattern of EBV expression in which transcripts from BamHI K, BamHI W and Y are detected. In addition, expression from EcoRI E is found in the majority of samples. NPC tissue demonstrates a greater complexity of EBV specific transcription, which we term "abortive". Transcripts corresponding to the latent open reading frames are detected as previously described. In addition BGLF1, the small EcoRI J RNAs and regions corresponding to BamHI B, BamHI I or A are also frequently transcribed in NPC biospy material.

S18.3
HERPES SIMPLEX VIRUS LATENCY IN VITRO
C.M. Preston and J. Russell, Institute of Virology, Church St., Glasgow, Scotland.

A tissue culture model which attempts to reproduce the phenomenon of herpes simplex virus latency will be described. Human foetal lung (HFL) cells are infected at the supraoptimal temperature of 42° and maintained at this temperature for 6 days. Cell cultures can then be returned to the permissive temperature of 37° without the subsequent appearance of HSV. Virus mutants have been used to show that only limited, if any, gene expression is necessary for establishment and maintenance of latency in vitro, and that the immediate early polypeptide Vmw110 is involved in reactivation. Current studies are aimed at understanding the state of the virus genome during latency in vitro.

S18.4
THE ROLES OF IMMEDIATE-EARLY PROTEINS IN REGULATION OF HERPES SIMPLEX VIRUS GENE EXPRESSION. P. Schaffer, Dana-Farber Cancer Institute and Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts, U.S.A. Herpes simplex virus encodes five immediate-early proteins designated infected cell polypeptides (ICPs) 0, 4, 22, 27, and 47 that play a role in the regulation of viral gene expression during productive and latent infections. Characterization of mutants generated by site-directed mutagenesis has revealed that ICPs 4 and 27 are essential for virus replication whereas ICPs 0, 22 and 47 are not. ICP4 is required at the transcriptional level for the expression of early and late genes and for repression of its own expression. ICP27 is required for the expression of late genes and may also play a role in the repression of immediate-early gene expression. In a mouse eye model for latency, two replication-deficient mutants in ICP4 established latency but could not be reactivated, whereas a replication-competent ICP4 mutant was able to be reactivated. These findings demonstrate that ICP4 is required for reactivation from latency consistent with its requirement in productive infection. Of interest was the observation that a replication-competent deletion mutant in ICP0 was not reactivated although viral DNA was readily detected in trigeminal ganglia. These findings suggest an essential role for ICP0 in the reactivation, but not the establishment, of latent infection.

S18.5
THE HERPES SIMPLEX VIRUS FUNCTION WHICH DESTABILIZES INFECTED CELL mRNAS. N. Frenkel, A.D. Kwong. Department of Molecular Genetics and Cell Biology, University of Chicago, Chicago, Illinois 60637.

Herpes simplex virus virions contain a function which shuts off host protein synthesis. A mutant in this function, vhs-1, exhibits altered phenotypes: (i)it is deficient in the rapid shutoff of host protein synthesis, (ii)it does not degrade host mRNA early post infection, (iii)it expresses the immediate early (α), early (β) and late (γ) viral proteins from stable mRNAs. In the wt virus infected cells the viral mRNAs are labile. All of these altered phenotypes co-map in a 265 bp fragment spanning coordinates 0.604-0.606. This region encodes a 58,000 dalton protein (Frink et al., J. Virol. 39: 559, 1981). Thus, the shutoff of host protein synthesis is mediated by a virion function which destabilizes both host and viral mRNAs. This function may allow the pattern of translation of infected cell proteins to directly reflect changes in the pattern of transcription of viral genes. Interestingly, in HSV-2, the equivalent gene contains the sequence shown to transform cells in culture (Galloway et al. Proc. Natl. Acad. Sci. 81:4736,1984). The proximity (or identity) of the transforming function to the mRNA destabilizing function may explain the selection of transformed cells which have lost the input transforming sequences, since the vhs function may not be compatible with cell survival.

S18.6
REGULATORY MECHANISMS IN HUMAN CYTOMEGALOVIRUS GENE EXPRESSION. E. S. Mocarski, R. R. Spaete and A. P. Geballe. Department of Medical Microbiology, Stanford University, Stanford, California.

Our studies on human CMV have indicated that the expression of the major β gene of CMV is regulated at the posttranscriptional level by signals carried on the β gene transcript. Our analytical studies have relied upon transient assays and the E. coli lacZ gene as an indicator to identify regulatory signals. We mapped the regulatory signal controlling gene expression at the posttranscriptional level to the 5' leader region of this gene and showed that it delayed full expression of the transcriptionally active gene. The transcript is present in the cytoplasm at times when it is not fully expressed, suggesting a block in translation. We have shown that transcripts containing the leader sequence are inefficiently translated in rabbit reticulocyte lysates and investigated the relative importance of short open reading frames and secondary structure in the leader. We have introduced lacZ under the control of this β gene control back into the CMV genome. Expression is consistent with our transient expression studies. Using lacZ as a genetic marker we have demonstrated that the CMV genome is amenable to site-directed insertion and deletion mutagenesis.

S19.1

REPLICATION OF VIROIDS. H.-P. Mühlbach, R. Kern, I.M. Schindler, M. Tabler, M. Tsagris and M.L. Sänger. Abteilung Viroidforschung, Max-Planck-Institut für Biochemie D-8033 Martinsried, Federal Republic of Germany.

Viroids are small single-stranded circular RNA molecules which cause diseases in higher plants. To study the molecular mechanism of viroid replication, we used the structurally well characterized potato spindle tuber viroid (PSTV) as a model. Our studies are focussed on the biochemical analysis of the replication intermediates, the temporal order of their synthesis in vivo, the subcellular location of the various forms of PSTV RNA occurring during replication, and on the host cell components involved in the synthesis and processing of PSTV RNA.

From time course analyses with PSTV-infected potato protoplasts, in vitro transcription studies with isolated nuclei, and from PSTV RNA processing experiments with nuclear extracts prepared from potato cells the following model of PSTV replication evolved: PSTV is replicated via concatameric RNA intermediates of (+) and (–) polarity. These intermediates are synthesized in the nucleus of infected cells by the DNA dependent RNA polymerase II. The monomeric circular PSTV progeny is finally generated from the concatameric PSTV(+)RNA strands by a cleavage and ligation process, for which nuclear proteins present in healthy potato cells are required.

S19.2

MUTAGENESIS OF INFECTIOUS POTATO SPINDLE TUBER VIROID cDNAs. R.W. Hammond, R. A. Owens, and T.O. Diener. Microbiology and Plant Pathology Laboratory, Beltsville Agricultural Research Center, USDA, Beltsville, MD, USA

Viroids are single-stranded, covalently closed circular RNAs that cause transmissible diseases in several economically important crop plants. Comparative sequence analysis and physical-chemical studies of these small, autonomously replicating pathogens have suggested that defined portions of their characteristic rod-like secondary structure control certain biological functions. We are using in vitro mutagenesis of infectious potato spindle tuber viroid (PSTV) cDNAs to examine the relationship between the structure of PSTV and its ability to replicate and incite disease.

Oligonucleotide-directed mutagenesis within the central conserved and pathogenicity domains has shown that slight modifications of the native structure can dramatically reduce the ability of PSTV to replicate systemically. Infectivity studies using cDNA chimeras and mixtures of mutant PSTV cDNAs or RNA transcripts also emphasize the role of the central conserved region in viroid replication. We are attempting to extend these observations by screening a large number of single-base substitution mutants generated by random chemical mutagenesis for their effects upon cDNA infectivity in a systemic tomato bioassay.

S19.3

STRUCTURE-FUNCTION ANALYSES USING INFECTIOUS TRANSCRIPTS FROM CLONED cDNAS OF CUCUMBER MOSAIC VIRAL SATELLITES. C.W. Collmer and J.H. Kaper. Microbiology and Plant Pathology Laboratory, Agricultural Research Service, U.S. Dept. of Agriculture, Beltsville, Maryland, U.S.A.

Complete cDNA copies of two variants of the cucumber mosaic virus satellite CARNA 5 (=CMV-Associated RNA 5) have been cloned in the transcription vector pPH1. These two naturally-occurring satellites are capable and incapable, respectively, of inducing a lethal necrotic disease of tomato upon coinoculation with the genomic RNAs 1, 2, and 3 of cucumber mosaic virus. Uncapped transcripts synthesized in vitro from the two linearized, recombinant plasmids are infectious and each induces appropriate symptoms upon coinfection with cucumber mosaic viral RNAs on tomato plants. Progeny CARNA 5s isolated from such infected plants correspond to their natural CARNA 5 counterparts. Specific structural features of the two cloned CARNA 5 variants, including start codons of potential open reading frames (ORFs), have been altered using site-specific mutagenesis. Transcripts from such mutants are being assayed for infectivity, stability, and ability to induce the tomato necrosis disease.

S19.4

STRUCTURAL FEATURES OF VIROID REPLICATIVE INTERMEDIATES AND OF SATELLITE RNAs OF CMV

Gerhard Steger, Inst. Physikal. Biologie, Universität Düsseldorf, Düsseldorf, FRG.

Different linear oligomeric viroid RNAs were synthesized as model substances for naturally occuring viroid replicative intermediates. The structural and dynamic features of these RNAs were studied by optical denaturation, temperature-gradient gel electrophoresis (TGGE), and thermodynamic calculations. In contrast to mature circular viroids, the intermediates are able to form inter- or intramolecularly a very stable secondary structure which allows the segmentation of the oligomeric molecules into structural units. This special structure may be the biological prerequisite of the cleavage and ligation to mature viroids. The existence of the structure in vivo will be shown by TGGE.

Sequence variants of the ds form of satellite RNAs of CMV were analyzed by TGGE in respect to the experimental detection of minor nucleotide exchanges. Melting of the dsRNAs was interpreted quantitatively on the basis of the Poland algorithm. A single base exchange may be detectable by TGGE, if it is located in a favorable region. Therefore, an experimental correlation between stability of parts of the double strand and necrogenicity was derived for all satellite variants under consideration.

S19.5

REVERSE GENETICS ANALYSIS OF THE SATELLITE RNA OF TOBACCO RINGSPOT VIRUS.

Jim Hasseloff and Wayne Gerlach

CSIRO Division of Plant Industry. Canberra. Australia.

The satellite of tobacco ringspot virus (STobRV) is member of a class of small (300-400b), single-strand RNAs which are dependent on a helper virus for propagation and which, in the absence of self-encoded polypeptide products, must rely for replication on RNA-encoded catalytic activities or contain signals for recognition by host plant or helper virus enzymes. Further, propagation of STobRV results in marked amelioration of symptoms caused by the host virus. We are using two "reverse-genetics" approaches to further define these activities and signals encoded by the STobRV RNA.

1. *In vitro* mutagenesis of STobRV.

From cloned STobRV cDNA, a plasmid for *in vitro* STobRV RNA transcription has been constructed and used to obtain a library of randomly mutagenized STobRV sequences. Screening of this library has allowed precise definition of the regions required for (+) and (–) strand autolytic RNA cleavage. Sequences required for replication are being similarly mapped. Interestingly, propagation of certain STobRV mutants gives rise to variant forms, presumably derived by recombination.

2. Expression of STobRV from the host plant genome.

Copies of STobRV cDNA have been integrated and expressed in tobacco plants to provide an alternative mechanism for the production and intercellular spread of STobRV in the absence of helper virus. In this way sole contributions of the host plant to STobRV replication can be characterized.

S19.6

SMALL RNA'S ASSOCIATED WITH BEET NECROTIC YELLOW VEIN VIRUS, AGENT OF RHIZOMANIA. S. Bouzoubaa+, H. Guilley+, G. Jonard+, D. Lemaire#, D. Merdinoglu#, C. Putz#, V. Ziegler+ and K. Richards+.

+ Laboratoire des Virus des Plantes, IBMC, Strasbourg, France
# Station de Pathologie Végétale, INRA, Colmar, France.

BNYVV has a multicomponent plus-strand RNA genome consisting of two long RNAs, RNA 1 (6746 nt) and RNA 2 (4612 nt), and two short RNAs, RNA 3 (1774 nt) and RNA 4 (1467 nt). Sequence homology among the four RNAs is limited to the 3' terminal 70 nt and a few nt at the 5' terminus. When propagated by mechanical inoculation to C. quinoa leaves RNAs 3 and 4 may undergo deletions or disappear. The presence of unit length RNA 3 is associated with increased symptom severity on leaves ; no effect of RNA 4 has been detected. In naturally infected sugarbeet root RNAs 3 and 4 of unit size are always present suggesting that the small RNAs, while behaving as satellites under the artificial conditions of leaf propagation, are essential for one or more steps in the natural infection cycle. Experiments aimed at determining the role of the small RNAs will be discussed.

S20.1

CLEAVAGE OF THE BACTERIOPHAGE P1 PACKAGING SITE (pac) IS REGULATED BY ADENINE METHYLATION. J. Coulby, N. Sternberg. E. I. duPont de Nemours & Co., Inc., Experimental Station, CR&DD, Wilmington, DE 19898 U.S.A.

Bacteriophage P1 packages DNA by a processive "headful" mechanism from a concatameric form of DNA. An initial step in that reaction is the recognition and cleavage of a unique P1 site (pac). We have been studying that step, both in vivo and in vitro, and have shown that: 1) Pac cleavage in vivo generates two DNA ends that are processed differently by the cell. Packaging is unidirectional and the end that would normally be packaged is protected from cellular nucleases. The other end is rapidly degraded by those nucleases. 2) Pac cleavage can occur in the absence of both viral capsid production and "late" protein synthesis. 3) The product of at least one P1 gene is essential for pac cleavage, and pac is located within the N-terminal portion of that gene. 4) A functional pac site consists of a 160 bp segment of DNA containing 4 dam methylation sequences (5'-GATC-3') at one end and 3 at the other. Neither hemimethylated or unmethylated pac sites are cleaved in an in vitro assay. 5) Cleavage of pac occurs over a single turn of the DNA helix in a region centrally located within the site.

Based on these and other observations (P1 encodes its own dam methylase analog) we will propose a model to account for the regulation of pac cleavage by DNA replication and adenine methylation of dam sites.

S20.2

TWO NOVEL MECHANISMS EXERTED BY THE BACTERIAL VIRUS T7 TO AVOID DNA RESTRICTION. D. H. Krüger[a], C. Schroeder[a], M. Reuter[a], T. A. Bickle[b], G. Barcak[c], M. O. Smith[c]. [a]Institute of Virology, Humboldt University, Berlin 1040,GDR; [b]University Biocenter, Basel 4056, Switzerland; [c]Johns Hopkins University Medical School, Baltimore, MD 21205, USA.

The *ocr* protein encoded by the bacterial virus T7 was the first protein identified which specifically blocks certain restriction endonucleases and DNA methylases (1). Moreover, there was a direct selection against the occurrence of methylation and/or cleavage sites in the viral DNA recognized by enzymes which are not inhibited by the *ocr* protein (2).

We have recently identified two novel mechanisms that also promote restriction avoidance in the T7 genome. The first case applies to the type III restriction enzyme *Eco*P15, which recognizes the non-symmetrical site, CAGCAG. Although the site is present 36 times in the viral DNA, cleavage does not occur. Protection is due to the fact that the methylatable adenine of the site is located only on the heavy strand of the DNA, i.e. there is absolute "strand bias". Second, the two *Eco*RII sites in T7 DNA are resistant to cleavage by the enzyme *in vivo* and *in vitro* because of their "low frequency occurrence" in the genome. Cleavable heterologous DNAs (e.g., pBR322 dcm$^+$) are able to "trans-activate" T7 DNA cleavage.

In addition, these results give insight into the mechanisms of action of these restriction endonucleases.

(1) Krüger & Bickle (1983), Microbiol. Rev. 47, 345-360.
(2) Schroeder et al. (1986), Gene 42, 77-86.

S20.3

FILAMENTOUS PHAGE ASSEMBLY. Marjorie Russel, The Rockefeller University, New York, NY 10021, U.S.A.

The filamentous phages (f1, M13, and fd) consist of a single-stranded circular DNA genome encapsidated in a long, slender protein sheath. Phage assembly occurs at the cytoplasmic membrane of infected cells, and progeny phage are extruded from the cell as they assemble. Unlike the spontaneous assembly pathways of the other (icosohedral) bacteriophages, filamentous phage assembly is a facilitated process. In addition to the five viral structural proteins, at least four additional components are required for efficient assembly in vivo: the single-stranded circular DNA molecules must contain a symmetry element called the morphogenetic or packaging signal, and the host-encoded protein, thioredoxin, plus two phage-encoded proteins (pI, pIV) that are not part of the virions, must be present.

We have been using a genetic approach to define the specific roles of these components, with special emphasis on the packaging signal and thioredoxin. Our results demonstrate that the redox activity of thioredoxin is not required for assembly. A detailed structure-function map of the packaging signal has been constructed. We have used phage lacking the packaging signal to select second site suppressors which affect genes encoding some of the components of assembly. These suppressors implicate the morphogenetic protein, pI, and the virion structural protein, pIX, in the initiation of phage assembly.

S20.4

STUDIES ON RNA COLIPHAGES, J. VanDuin, Dept. of Biochemistry, State University of Leiden, Leiden, Netherlands

ABSTRACT NOT AVAILABLE

S20.5

BACTERIOPHAVE LAMBDA AND ITS DEPENDANCE ON THE HEAT SHOCK PROTEINS OF Escherichia coli, C. Georgopoulos, Dept. of Cellular Viral and Molecular Biology, University of Utah Medical Center, Salt Lake City, UT, USA

ABSTRACT NOT AVAILABLE

S20.6

BACTERIOPHAGE LAMBDA DNA TERMINASE - RECENT STUDIES. H. Murialdo, Dept. of Medical Genetics, University of Toronto, Toronto, Ontario, Canada

Lambda DNA terminase is composed of two subunits called Nul and A. The enzyme recognizes a specific sequence called cosB and introduces two staggered nicks, twelve base pairs apart, one on each of the two complementary DNA strands. The cutting site is called cosN. About 100 molecules of enzyme are produced per phage infected cell. This low productivity has hampered the study of its binding sites and mechanism of action. We have recently cloned the two genes coding for the subunits of the terminase and succeeded in expressing them with the production of large amounts of protein. A plasmid has been constructed that produces more than a thousand-fold more enzyme than phage infected cells. We are now in the process of purifying large amounts of enzyme to study its binding sites to DNA, its interaction with other proteins and, most importantly, its crystallographic structure. Recent data on the above characterization of the lambda DNA terminase will be discussed.

S21.1

REQUIREMENTS FOR VIRUS DIAGNOSIS BASED ON ANTIGEN DETECTION. C R Madeley. Department of Virology, University of Newcastle upon Tyne, Royal Victoria Infirmary, Newcastle upon Tyne NE1 4LP, United Kingdom.

Any detection system is only as good as the specimens provided. Provision of good specimens requires active and continuing encouragement by laboratory staff and attention to methods of transporting without delay and at suitable temperature is vital. Quantity of specimen is directly related to success and it must be collected from the best area. This depends on an understanding of disease processes and the diagnostic test to be used. For example, respiratory virus diagnosis by immunofluorescence (IF) depends on the collection of respiratory rather than squamous cells in nasopharyngeal secretions or broncho-alveolar lavage.

Direct antigen detection may include visualizing complete virus (EM), detecting infected cells (IF), detecting viral antigen (immunoassays (enzyme-, radio- etc.), TR-FIA, latex agglutination, etc.). Where they depend on polyclonal antisera, care in preparing them and proving their specificity is difficult, tedious, expensive and vital. Monoclonal antibodies should be more specific but this must be proved on clinical specimens before routine use. For acute specimens the sensitivity of a test may be less important than getting the right specimen but for screening (for HIV or hepatitis B, for example) this can present considerable problems.

S21.2

SCREENING AND EVALUATION OF MONOCLONAL ANTIBODIES FOR VIRAL ANTIGEN DETECTION. Monica Grandien, Department of Virology, National Bacteriological Laboratory, S-105 21 Stockholm, Sweden.

The potential of monoclonal antibodies, Mab, as reagents for diagnostic virology, can be fully exploited only after careful characterization and evaluation. - After primary screening of specificity of the hybridoma cell product with a general test e.g. ELISA or immunofluorescence, basic properties such as homogenicity, stability and isotype should be determined. Knowledge of the precise polypeptide target is gained by the radioimmune precipitation assay. Additional information may be obtained by analyses of biological activities.

The antigen target specificity should be selected in accordance with the test principle and the Mab chosen should be directed against antigens most abundantly present in infected material. When a test system has been chosen, the Mab affinity and antigen binding capacity should be ascertained. Mab reactions with a reasonable number of wild virus strains, preferably from different geographical areas, should be investigated.

The choice of a Mab with specificity to conserved or strain specific epitopes is dictated by the aim of the investigation.

S21.3

EXPERIENCE FROM VIRAL ANTIGEN DETECTION BY ENZYME IMMUNOASSAY IN DAILY DIAGNOSIS. T. Ziegler, O. Meurman, P. Halonen. Department of Virology, University of Turku, Turku, Finland.

Detection of viral antigens from clinical specimens by enzyme immunoassay (EIA) provides a specific and sensitive tool for rapid diagnosis of viral infections. For several years such assays have been in routine use of our laboratory for the detection of antigens of ten different viruses: RSV, influenza A and B, parainfluenza 1,2 and 3, rotavirus, adenovirus, HSV and VZV. When evaluating against virus isolation or IgG serology on serum pairs, antigen detection is slightly less efficient in diagnosing respiratory infections than the reference methods. In all comparative studies, however, collection and transportation of specimens for virus isolation have been optimized to a degree as it is not possible under routine conditions. The availability of the results within 24 hours and the fact that one technician can screen approximately 3,000 nasopharyngeal secretions for seven different viruses per year clearly outweigh the slightly decreased sensitivity of antigen detection.

Antigen detection is useful for diagnosing vesicular rashes caused by HSV or VZV in patients who may benefit from antiviral therapy. However, for the diagnosis of genital HSV infections, our EIA cannot compete with virus isolation.

In contrast to immunofluorescent staining the specimen for EIA is thoroughly homogenized before testing. This allows the performance of control tests on identical material.

S21.4

ANTIGEN DETECTION BY ENZYME IMMUNOASSAY USING A VACUUM FILTRATION SYSTEM. D.D. Richman, P.H. Cleveland. Departments of Pathology, Medicine and Ophthalmology, University of California San Diego and San Diego V A Medical Center, USA.

A simple, sensitive, 30-min assay for antigen detection has been developed utilizing a filter manifold, biotinylated monoclonal antibodies, streptavidin-enzyme complex, and a substrate yielding a precipitable colored product. With reconstruction experiments, as few as two HSV or VZV infected cells can be detected, which is 100 fold more sensitive than enzyme immunoassays that detect a soluble reaction product.

The technique has proven rapid, sensitive and specific for culture confirmation of herpes viruses, adenovirus, Lassa virus, dengue virus types 1-4, HIV and several respiratory viruses. For direct, rapid detection in clinical specimens, 66 (93%) of 71 HSV culture-positive specimens and all 9 (100%) of VZV culture positive specimens were positive. The application to studies of HSV shedding from the genital tracts of women studied in STD and pregnancy clinics will be presented. In addition, the assay was 83% sensitive compared to culture when applied to an outbreak of epidemic adenovirus keratoconjunctivitis.

S21.5

NEW CONCEPTS IN DIAGNOSIS OF INFLUENZA BY RAPID CULTURE CONFIRMATION WITH TYPE-SPECIFIC MONOCLONAL ANTIBODIES. A. P. Kendal. W.H.O. Collaborating Center for Influenza, Centers for Disease Control, Atlanta, GA. USA. The traditional approaches to influenza virus diagnosis are usually too slow to be useful for patient treatment or outbreak control. Alternative procedures for diagnosis, based on direct antigen detection, cannot always be undertaken due to physicians or patients balking at the collection of specimens other than throat or nose swabs. Culture confirmation has now been shown capable of yielding rapid results with high sensitivity and specificity, when monoclonal antibodies (MAB) specific for conserved antigens in influenza A or B viruses are used to detect virus growth. Test formats for culture confirmation include (1) direct or indirect fluorescent antibody staining on tube cultures, coverslip cultures or microscope slide cultures, (2) enzyme immunoassays, or (3) time-resolved immunofluorescence. In various studies, many virus-containing specimens grew detectable levels of antigen after overnight incubation of infected cells, and consistently 80% or more of virus-containing specimens were confirmed as growing virus within 2 or 3 days. During epidemic periods about one-third of specimens collected by sentinel physicians and sent by mail to the laboratory have yielded positive culture confirmation results in under a week, including transport time. Based on these recent reports, it is anticipated that the fastest and most economical culture confirmation test(s) will displace traditional procedures in most virology laboratories which provide primary diagnostic services for influenza. Isolates can be forwarded to State and National reference laboratories for further analysis, such as hemagglutinin sub-typing.

S22.1

THE S1 NUCLEOTIDE SEQUENCES OF SEVEN FIELD ISOLATES OF TYPE 3 REOVIRUS. T. Dermody, M. Nibert, R. Bassel-Duby, B. Fields. Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, MA USA

Many biologic properties important in reovirus pathogenesis (spread, tissue tropism, cell attachment and interaction with the immune system) map to the S1 gene. In order to gain further insight into structure-function relationships of the σ1 protein, we have determined the S1 nucleotide sequences of seven field isolates of type 3 reovirus. Nucleotide sequence analysis reveals limited heterogeneity in the predicted σ1 amino acid sequences. The small number of changes observed between the different isolates have allowed us to define a specific site important for the σ1-associated phenotype of hemagglutination and a region critical for stability of the σ1 multimer in SDS-polyacrylamide gels.

S22.2

VIRAL PROTEIN CLEAVAGE SITE TO MAP PATHOGENESIS AND RAISE VACCINES. M. Homma, M. Tashiro*, M. Itoh. Department of Microbiology, Kobe University School of Medicine, Kobe and Department of Bacteriology, Yamagata University School of Medicine, Yamagata*, Japan.

Cleavage of the envelope glycoprotein (F) of Sendai virus is mediated by a trypsin-like host protease and enables the virus to penetrate the cells. Thus the presence of the protease in the host will determine the host range and organ tropism as well. A protease-activation mutant, TR, is resistant to trypsin but can be activated by chymotrypsin. When chymotrypsin-activated TR is administered into mice, replication of the progeny virus occurs in the bronchiolar epithelium but only in a single cycle, resulting in no appreciable lung disease. The animal, however, completely tolerates the challenge with the wild type virulent virus. The F gene analysis of TR shows a single amino acid substitution of Ile for Arg at the position 116, the site at which trypsin works and cleaves F protein of the wild type virus. The above results reveal that one point mutation is enough to determine the pneumopathogenesis of Sendai virus. An applicability of TR as a live vaccine is presented.

S22.3

MOLECULAR AND IMMUNOLOGIC BASIS OF CNS VIRULENCE OF MOUSE HEPATITIS VIRUS-4 (MHV-4). M.J. Buchmeier. Research Institute of Scripps Clinic, La Jolla, CA 92037.

Most mice infected intracerebrally with wild type MHV-4 die in 5-7 days due to acute encephalomyelitis. In the small fraction of animals surviving acute infection CNS demyelination is evident. Several laboratories have shown that neurons are spared in the attenuated disease but infection of oligodendrocytes, the cell which forms and maintains myelin sheaths, occurs. Our laboratory has attempted to define the host and viral factor(s) which determine neurotropism and limit the spread of infection in vivo in the CNS. We have used monoclonal antibodies (MAb) of defined specificity in a passive transfer model to define viral proteins which are important determinants of neurovirulence. Using this approach we have shown that MAb against two distinct epitopes on the MHV-4 E2 peplomer glycoprotein blocked development of fatal encephalitis following ic infection with wild type virus. Although neurons were spared, oligodendrocytes were infected and demyelination resulted. Having shown that sites on E2 were involved, we selected variant strains of MHV-4 with mutations in E2 which were resistant to E2 directed neutralizing MAb. These mutant strains of virus were attenuated by a factor of 2-3 logs in LD_{50}, but continued to induce chronic demyelinating disease characterized by inflammation, primary demyelination, remyelination and recurrent demyelination over a prolonged period, directly demonstrating that the E2 glycoprotein is an important determinant of neurovirulence. Further studies are directed toward identifying the nature of lesions in the variants, their effect on tropism and virulence, and specificity of host responses to viral and self components.

S22.4

VIRUS INDUCED AUTOIMMUNITY. A. Notkins, National Institute of Health, Bethesda, MD, USA

ABSTRACT NOT AVAILABLE

S22.5

IMMUNOLOGY AND IMMUNOPATHOLOGY OF HUMAN HERPES VIRUS INFECTIONS - WITH REFERENCE TO CMV. J.G.P. Sissons, L.K. Borysiewicz, J.K. Hickling, D. Scott, M. Crannage*, G.A. Smith*. Dept. Medicine, Royal Postgrad. Medical School, London UK. and *Division of Virology, Dept. Pathology, Cambridge UK.

Human cytomegalovirus (HCMV) gene expression occurs in three sequential phases: HCMV immediate early (IE) genes may be the only virus genes expressed in cells non permissive for lytic infection, and in a small fraction of peripheral blood mononuclear cells during HCMV persistence in the normal host. We have used recombinant vaccinia viruses expressing the genes for the HCMV major IE protein (mIE)and for glycoprotein B (gB - a late protein), coupled with limiting dilution analysis, to assess the relative frequencies of class I restricted cytotoxic T cells (Tc) recognising these different stage specific HCMV gene products. Results show that in normal HCMV carriers a much higher proportion of clonally derived Tc generated in response to HCMV are specific for mIE than for gB. Whether HCMV IE is a dominant antigen for all individuals and which portions of the molecule are relevant peptide epitopes remains to be determined. During murine CMV persistence U. Koszinowski and colleagues have previously shown a major proportion of the protective Tc response is directed to IE antigens. The events regulating IE gene expression, the sites involved, and the IE specific Tc response may be important determinants of the pathogenesis of HCMV.

S23.1

GENOMIC ORGANISATION OF GEMINIVIRUSES INFECTING DICOTYLEDONOUS HOSTS. A. Ward, J. Stanley, R. Townsend, P. Ettesami, K. J. Kunert. Dept. of Virology, John Innes Institute, NORWICH, England

The cassava latent virus (CLV) genome comprises two small (approx. 2.7Kb), ssDNA circles, both of which are required for systemic infection of plants. Transcript mapping indicates that six ORFs which are strongly conserved between closely related geminiviruses, are most likely to be functional.

DNA-1 was shown to replicate autonomously in protoplasts. The coat protein, encoded on its virion sense, has been found to be unnecessary for systemic infection of plants. This suggests that the three complementary sense ORFs might encode the viral replicative functions.

DNA-2 encoded products are implicated in systemic spread of the virus. Mutations made in either of its ORFs are lethal, implying both are essential for virus spread.

Chimaeric forms of the virus are being used to investigate the possibility that intergenic sequences are involved in the control of viral gene expression and replication. These experiments have a bearing on the potential use of geminiviruses as gene vectors.

S23.2

MAIZE STREAK VIRUS, ITS REPLICATION AND THE PROSPECTS FOR ITS USE AS A VECTOR FOR THE TRANSFORMATION OF MONOCOTYLEDONOUS PLANTS. M.I. Boulton, M.S. Marks, W.R. McLauchlan, P.D. Bowyer, J. Donson, G.P. Harrison, P.G. Markham, P.M. Mullineaux and J.W. Davies, Department of Virus Research, John Innes Institute, Colney Lane, Norwich NR4 7UH, U.K.

Agrobacterium-mediated virus infection - "agroinfection" has allowed the systemic infection of maize plants by cloned maize streak virus (MSV) DNA. A dimer has been constructed from a tandemly repeated monomeric unit of the MSV genome and inserted into the binary T DNA vector Bin 19. When this was mobilised into selected strains of Agrobacterium and inoculated into maize plants, the resultant infection was identical both in symptomatology and by electron microscopic examination to that obtained following virus transmission by the leafhopper vector, thus confirming the monopartite nature of the MSV genome. The mode of introduction of the DNA into the plant and the implications of the technique for the tranformation of graminaceous monocotyledons will be discussed.

Deletion mutants and/or insertional mutants for each of the potential open reading frames of MSV have been constructed. Several mutants containing marker genes are also under investigation, for example a mutant containing the neomycin phosphotransferase gene of Tn5 in place of the coat protein gene has been introduced into maize plants and protoplasts. The results obtained following the inoculation of all these mutants will be presented.

S23.3

PROPAGATION AND EXPRESSION OF FOREIGN GENES IN CELLS OF MONOCOTYLEDONOUS PLANTS BY A RECOMBINANT WHEAT DWARF VIRUS.

V. Matzeit, U. Commandeur, M. Kammann, J. Schalk, S.Schaefer, B. Gronenborn
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The genomes of two different isolates of wheat dwarf virus (WDV), a geminivirus with a single circular single-stranded DNA genome, were cloned and sequenced. The chromosome of an isolate originating from Sweden consists of 2749 bases, that of an isolate from Czechoslovakia has a size of 2751 bases. The genome organisation of the two virus isolates is very similar.
Cloned DNA of both isolates was used for the infection of protoplasts of *Triticum monococcum* and *Zea mays* suspension culture cells. All the different replicative and virion associated forms of WDV DNA were found in the infected cells.
Mutant viral genomes were constructed *in vitro* replacing the capsid protein gene of WDV by the neomycin phosphotransferase, the chloramphenicol acetyltransferase or the ß-galactosidase gene of *E. coli*. All three different foreign genes are replicated and expressed by the wheat dwarf virus vector.

S23.4

REGULATION IN CAULIFLOWER MOSAIC VIRUS. Th. Hohn, J.-M. Bonneville, J. Fütterer, K. Gordon, J. Martinez-Izquierdo, J. Penswick, B. Pisan, H. Sanfaçon. Friedrich Miescher-Institut, P.O.Box 2543, CH-4002 Basel, Switzerland.

Several viruses had provided excellent model systems to study transcriptional control. Cauliflower Mosaic Virus, a plant reversovirus, which produces a large, apparently polycistronic RNA with terminal repeats and a long leader, provide a good model to study post-transcriptional control mechanisms. While this RNA and derivatives of it are difficult to translate in either wheat germ or reticulocyte in vitro systems, translation can be studied employing transient expression of reporter coding regions in host and on-host protoplasts. Preliminary results indicate post-transcriptional transactivation by both, host and virus coded factors.

Protein processing and viral autophosphorylation are candidates for control mechanisms on a different level, namely enzyme activation and virus assembly.

S23.5

FUNCTION AND EXPRESSION OF GENINIVIRUS GENES IN TRANSFORMED PLANTS. D.M. Bisaro,§ S.G. Rogers,• G.Sunter, W.E. Gardiner, J.S. Elmer,• L. Brand,• I. Chang, C.K. Browning. Department of Botany and Microbiology, Auburn University, Auburn, AL 36849 and •Biological Sciences, Monsanto Company, St. Louis, MO 63198, USA.

Tomato golden mosaic virus (TGMV), a whitefly-transmitted geminivirus, has a genome composed of two circular ssDNAs referred to as the A and B components. Both DNAs, each ~2.5 kb in size, are necessary for infectivity.

We have investigated the functions encoded by each of the TGMV genome components using the Agrobacterium tumefaciens Ti plasmid gene transfer system. Genetic and biochemical analysis of transgenic plants containing TGMV-DNAs has shown that the A component encodes all viral information necessary for the replication and encapsidation of TGMV DNA. The B component specifies functions essential for systemic spread and subsequent symptom development. Further, the A-DNA encoded TGMV coat protein is not required for viral DNA replication, systemic spread or pathogenesis, although coat protein mutants do exhibit attenuated disease symptoms. Experiments are now in progress to define the roles of remaining five TGMV genes in virus multiplication.

S23.6

COINFECTION WITH HETEROLOGOUS CAULIFLOWER MOSAIC VIRUS (CaMV) DNA MOLECULES : RECOMBINATION AND ITS IMPLICATIONS FOR GENE TRANSFER WITH CaMV. A. Geldreich, H. Albrecht, M. Giband, D. Kirchherr, J. Ménissier-de Murcia, J.M. Mesnard, L. Hirth, G. Lebeurier. Laboratoire des Virus des Plantes, IBMC, 15, rue Descartes 67084 Strasbourg-Cédex, France.

Cauliflower mosaic virus (CaMV) is a small DNA virus with a double-stranded genome of 8000 bp. Sequencing of the genome has revealed six long and two small open reading regions as potential genes. A 35 S RNA covering the entire viral genome is used for reverse transcription and probably for translation. We demonstrate that in vivo an intergenomic recombination may be explained by dimerization of two heterologous CaMV DNA molecules and transcription into a hybrid 35 S RNA responsible for replication of the recombinant genome. The efficiency of this process indicates that transfer of a foreign gene to sensitive plants will probably require integration between genes I to V of one of the heterologous CaMV DNA molecules. Reasons for this hypothesis will be discussed.

S24.1

INTEGRATION OF AVIAN RETROVIRAL DNA: BIOCHEMICAL STUDIES. A.M. Skalka, D. Cobrinik[1], F. Alexander, R.A. Katz, D. Soltis and J. Leis[1], Department of Molecular Oncology, Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ 07110; [1]Department of Biochemistry, Case Western Reserve University, Cleveland, OH 44106.

Reverse transcription and integration of retroviral DNA into the host genome requires the function of the retroviral pol gene. Integration is site-specific for sequences on the retroviral DNA. Genetic studies have shown that the 3' terminal one-third of the pol gene, which encodes an endonuclease domain, is required for retroviral DNA integration. In the avian retrovirus, both the reverse transcriptase ($\alpha\beta$ form) and a processed protein (pp32) contain the endonuclease domain. Studies with these pol-endos have revealed preferred sites of cleavage that map to the unique U5/U3 junction found only in covalently closed circular DNA molecules containing two tandem viral LTRs. The cuts occur in either strand, 2 or 3 nucleotides 3' to the axis of symmetry of a 12/15 base pair inverted repeat at the junction, the region which is known to be joined to host DNA during integrative recombination. Deletion mutagenesis studies have defined a domain of 30-40 base pairs which is required for the site-selective cleavage and also for viral growth. Further biochemical and genetic studies of virion proteins as well as recombinant proteins produced in heterologous expression systems should reveal additional details concerning the mechanism of this important recombination event.

S24.2

TRANSLATIONAL CONTROL OF RETROVIRAL PROTEIN EXPRESSION AND FUNCTION. S. Oroszlan, BRI-Basic Research Program, NCI-Frederick Cancer Research Facility, Frederick, MD, U.S.A.

Retroviruses synthesize the internal structural proteins (*gag*-products) and replicative enzymes (*pol*-products) in the form of overlapping polyprotein precursors via the translation of the genome-sized mRNA. The viral protease is responsible for the processing of the precursors into the mature forms. Protein and nucleic acid sequencing accurately delineated the biosynthetic and proteolytic processes. In avian retroviruses the protease (*pro*) is located within the *gag* gene. It is however a part of the *pol* gene in mammalian type C viruses (*gag* and *pol* in same frame) and in lentiviruses where *pol* is translated in -1 frame relative to *gag*. In the bovine leukemia/human T-cell leukemia virus family as well as the type B and type D retroviruses the protease is encoded in a separate reading frame between the *gag* and *pol* genes and overlaps both. The expression of *pro* and *pol* genes is translationally controlled. Initiated at the same AUG codon of the 5' *gag*, the *gag-pro* and *gag-pro-pol* polyproteins are produced by suppression of the termination codons either by in-frame readthrough or frameshift. Several examples of both will be described and the most likely mechanisms discussed.

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S24.3

STRUCTURAL FEATURES OF RETROVIRAL GLYCOPROTEINS. E. Hunter, L. Perez, G. Davis, J. Dong and D. Miller. Dept. Microbiol., Univ. of Alabama at Birmingham, Birmingham, AL 35294.

The highly glycosylated Rous sarcoma virus env product undergoes proteolytic processing during transport to the plasma membrane; the precursor (Pr95) being cleaved to the mature virion proteins (gp85 and gp37) in the Golgi. The complex appears in the virion as a knobbed spike, the knob being comprised of gp85 and the spike of gp37. The glycoprotein has three functional domains: an external receptor-binding domain, a hydrophobic membrane-spanning anchor domain and a hydrophilic cytoplasmic 'tail'. We have explored the structural requirements for biosynthesis, processing and assembly into virions of this protein by constructing, in vitro, mutated envelope genes and by expressing such genes from an SV40 vector in primate cells or from an avian retrovirus genome in avian cells.

These studies show that the cytoplasmic domain is not required for normal transport or function of the env product; proviruses encoding a 'tail(-)'-mutant env gene are infectious and yield virions containing normal levels of glycoprotein. The hydrophobic domain alone is required to anchor the glycoprotein complex in the membrane and appears to play the critical role in recognition and assembly of the env complex into virions. Cleavage of Pr95 occurs after a series of basic amino acids: arg-arg-lys-arg. Mutation of the lysine to glutamic acid (mutant G1) or deletion of the sequence encoding these 4 a.a. (mutant Dr1) blocks cleavage 90 and 100% respectively. Neither mutation prevents transport to the cell surface but proviruses encoding the Dr1 protein are non-infectious. The role of Pr95 cleavage in viral assembly and infectivity will be discussed.

S24.4

HUMAN ENDOGENOUS RETROVIRAL ACTIVITY IN EMBRYOS AND TUMORS A. Vaheri, R. Pakkanen, O. Turunen, J. Suni and T. Wahlström Departments of Virology and Pathology, University of Helsinki, 00290 HELSINKI, Finland

In previous studies, using immunological reagents specific for animal retrovirus, we detected retrovirus-related IgG antibodies in human cord blood sera and retrovirus-related polypeptide antigens in human reproductive tissues and tumors. Using antibodies to a synthetic decapeptide deduced from a cloned human endogenous retroviral sequence we identified a M_r 75 000 protein in human placental syncytiotrophoblasts and hypernephroma. The M_r 75 000 protein was purified using several steps of HPLC. This protein is confined to the cytoplasmic side of microvillar plasma membrane of cultured choriocarcinoma cells. In an immunohistologic study the M_r 75 000 protein (designated as "cytovillin") was found in certain differentiating human embryonic tissues and reappeared in hypernephromas and some other tumors but was not detected in normal adult tissues. The cytovillin cDNA has been cloned from a placental lambda gt11 expression vector library and partially sequenced.

S24.5

TRANSCRIPTIONAL AND POST-TRANSCRIPTIONAL REGULATION IN REPLICATION OF HTLV. M. Yoshida, J. Inoue, J. Fujisawa and M. Seiki. Department of Viral Oncology, Cancer Institute, Toshima-ku, Tokyo, Japan.

Human T-cell leukemia virus type 1 (HTLV-1) is an etiologic agent of adult T-cell leukemia (ATL). The viral genome has a unique sequence so called "pX" between the env and 3' LTR, which codes for three proteins: p40(x), p27(x-III) and p27(x-III). The p40(x) is a transcriptional trans-activator, which activates the expression of the viral genome, thus essential for efficient viral gene expression. The p40(x) also activates cellular interleukin-2 and interleukin-2 receptor, thus thought to be involved in early stage of leukemogenesis. The second pX protein, p27(x-III), is a post-transcriptional modulator and required for gag and probably env protein expression elevating the gag and env mRNA. Therefore, balanced expression of p40(x) and p27(x-III) is indispensable for efficient viral replication. Significance of these two functions in viral replication will be discussed.

S24.6

TRANSDUCTION OF PROTO-ONCOGENES BY RETROVIRUSES L.-H. Wang. The Rockefeller University, New York, N.Y. USA

Evidence from sequence analyses of viral and cellular oncogenes suggests that incorporation of a proto-oncogene (c-onc) into a retroviral genome requires at least two steps of recombination.

The initial recombination between a viral genome and the 5' region of c-onc appears to occur at the DNA level. This step does not require extensive sequence homology and can be mediated by stretches of sequences with only partial homology. The 5' recombination junction can also be formed by splicing between viral and c-onc sequences. The second recombination is presumed to occur between the transducing viral RNA and the viral-c-onc hybrid RNA molecule generated from the initial recombination. This step involving recombination at the 3' ends of those molecules restores the 3' viral sequences essential for replication to the viral-c-onc hybrid molecule. The second recombination is greatly enhanced when there is sequence homology between the transducing virus and the 3' region of c-onc.

Incorporation of a c-onc sequence into the retroviral genome results in greatly elevated expression of the gene. However, increased expression of c-onc alone is often insufficient to activate its transforming potential. Structural changes in c-onc are usually necessary to convert it into a transforming gene.

S25.1

THE MOLECULAR BASIS OF REOVIRUS ATTACHMENT. Patrick W.K. Lee. Department of Microbiology and Infectious Diseases, Faculty of Medicine, University of Calgary, Calgary, Canada.

The reovirus attachment mechanism was probed through studies on the viral cell attachment protein (protein σ1), the gene (gene S1) from which it is derived, as well as the cellular receptor. The S1 gene has been cloned, sequenced and expressed in E. coli to yield a functional σ1 fusion protein capable of attaching to host cells (mouse L fibroblasts) and agglutinating erythrocytes. Studies on deletion mutants of the cloned S1 gene have shown that the domain responsible for host cell attachment is distinct from that controlling hemagglutination. Further manipulations of the cloned gene have revealed a site on the C-terminal half of the protein that is important for σ1 attachment. This information, coupled with functional analysis of biochemically fragmentized σ1, have allowed us to localize a stretch of amino acids on σ1 that may be involved in receptor recognition. The reovirus receptor on human erythrocytes has been identified as the major membrane sialoglycoprotein, glycophorin, whereas the receptor on mouse fibroblasts appears to be a 67K glycoprotein. Our data also clearly indicate that sugars on these receptors are important recognition signals for reovirus attachment and that both sialic acid and N-acetylglucosamine may be involved.

S25.2

ANTIBODY INHIBITS THE NEURAL SPREAD OF REOVIRUS TYPE 3 DEARING. K. Tyler, H. Virgin, R. Bassel-Duby, B. Fields. Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, MA USA

The capacity of Reovirus Type 3 Dearing (T3) to spread to the CNS via nerves in the infected host is determined by the S1 dsRNA segment, which encodes the σ1 protein. We have begun to investigate the effect of passive transfer of anti-T3sigma 1 monoclonal antibodies (MAbs) on the pathogenesis of T3 CNS infection. MAbs given (IP) simultaneously with or 24 hrs. prior to footpad challenge of neonatal mice with T3 (>100LD_{50}) protect mice, and inhibit the entry of virus into the spinal cord and brain without affecting its primary replication in skeletal muscle. MAbs given after T3 has already reached the inferior spinal cord also protect mice, and inhibit the spread of T3 from the inferior spinal cord to the superior spinal cord and brain. MAbs given 24 hrs. after intracerebral inoculation of T3 (>100LD_{50}) protect mice, inhibit CNS replication of T3, and block the centrifugal spread of T3 from the brain to the spinal cord and eye. These studies indicate that MAbs can protect mice against lethal challenge with a neurally spreading virus and illustrate several mechanisms for this protection: (1) inhibition of viral entry into the CNS, (2) inhibition of viral replication within the CNS, and (3) inhibition of spread of virus within the CNS.

S25.3

THE STRUCTURE AND FUNCTION OF REOVIRUS GENES W. K. Joklik, J. Wiener, J. Bartlett and J. B. Antczak. Department of Microbiology and Immunology, Duke University Medical Center, Durham, North Carolina, USA

Many of the reovirus genome segments have now been sequenced. This knowledge not only provides insight into the structure and function of the proteins that they encode, the manner in which the various reovirus serotypes have evolved and the relationship of reoviruses to other members of the Reoviridae family such as the rotaviruses and orbiviruses, but also provides information that is essential for cloning reovirus genes into expression vectors, carrying out site-directed mutagenesis, and characterizing their functional domains.

The sequences of the four S genes have been available for some time. All three M genes have now also been sequenced. Gene M3 encodes the nonstructural protein μNS which, like σNS, possesses a high α helix content and possess several repeated sequence elements; interestingly, several lines of evidence suggest a close association in the cell of μNS and the cytoskeletal network. Gene M2 encodes the major outer capsid shell component μ1C; all three forms of this gene have been sequenced. Gene M1 encodes the minor core component μ2 which has a much higher α helix content than bona fide structural proteins. We have also sequenced the L3 (protein λ1) gene and are in the process of sequencing the L1 and L2 genes, all of reovirus serotype 3. Details of the structure of protein λ1 will be presented.

S25.4

ANALYSIS OF ROTAVIRUS ANTIGENS BY GENE CLONING, MUTATION AND EXPRESSION. G.W. Both* and A.R. Bellamy**, *Division of Molecular Biology, CSIRO, Sydney, Australia and **Department of Cell Biology, University of Auckland, Auckland, New Zealand.

The importance of acute gastroenteritis as a public health problem and the unusual morphogenetic pathway of rotaviruses are two reasons why these viruses are now being intensively studied. A large number of animal and human rotavirus genes have been cloned in several laboratories. Of particular interest are those coding for the viral surface antigens VP3 and VP7, both because of their potential use in a molecular vaccine and because of the information their sequence determination has provided about the replication of the virus. Present indications are that most structural viral proteins enter the endoplasmic reticulum (ER) as part of a budding subviral particle. Only VP7 has a signal peptide which enables it to enter the ER independently. Mutation and expression of the gene has enabled us to determine the location of signal peptide sequences in VP7 and the site at which the protein is cleaved during translocation into the ER. The means by which VP7 is retained in the ER for incorporation into mature virus particles is also under investigation.

S25.5

MOLECULAR CHARACTERISATION OF ATYPICAL ROTAVIRUSES. M. A. McCrae[1] and J. C. Bridger[2]. [1]Department of Biological Sciences, University of Warwick, Coventry, CV4 7AL, England. [2]Institute for Research on Animal Diseases, Compton, Nr. Newbury, England.

Rotaviruses which fail to react in a number of serological assays detecting the previously defined group antigen have now been isolated from a range of animal species including humans, calves, lambs, pigs and chickens. Isolates of this type have been given various names but to simplify the nomenclature and classification of rotaviruses we have proposed a scheme in which these viruses are divided in distinct groups. Group A is reserved for viruses carrying the previously recognised rotavirus group antigen; groups B, C, D etc. contain the various atypical viruses. Experimental criteria using both serological and nucleic acid based assays have been established which allow new atypical rotavirus isolates to be assigned either to one of the existing five groups or designated as the type member of a new group. To circumvent problems with the in vitro propagation of these viruses we have been applying recombinant DNA technology to various non-group A viruses to facilitate their characterisation and provide reagents for use in diagnostic assays. The progress of the work to-date will be discussed as will the potential significance of the various non-group A isolates to the overall problem of acute viral gastroenteritis.

S25.6

DOUBLE-STRANDED RNA VIRUSES ASSOCIATED WITH PLANTS AND PLANT PATHOGENIC FUNGI. D. L. Nuss. Roche Institute of Molecular Biology, Roche Research Center, Nutley, New Jersey 07110 U.S.A.

Symptoms resulting from infection of plant hosts by double-stranded (ds)RNA viruses range from inapparent to neoplasia. Plant virus members of the Reoviridae, e.g., wound tumor virus (WTV) also exhibit the interesting property of replicating in their insect vectors. Double-stranded RNA viruses associated with plant pathogenic fungi can dramatically alter plant-pathogen interaction, i.e. virulence of the fungal pathogen. In the case of *Endothia parasitica*, the cause of chestnut blight, dsRNAs are thought to be responsible for conversion of virulent strains to hypovirulence providing a basis for biological control of the disease. Although, dsRNA genetic elements can have important biological consequences for plants and their fungal pathogens, only recently has there been much progress in elucidating the molecular biology of these elements. To illustrate the range of properties exhibited by dsRNA genetic elements found in plants and plant pathogenic fungi, the structural and functional properties of the dsRNA genome of WTV and the dsRNAs associated with hypovirulent strain of *E. parasitica* are examined.

S25.7

GENE EXPRESSION OF BIRNAVIRUSES. P. Dobos and R. Duncan. Department of Microbiology; University of Guelph, Canada.

Birnaviruses are medium-sized, naked, icosahedral viruses containing bisegmented dsRNA genomes (segment A ~ 3 Kbp and segment ~ 2.7 Kbp). The best studied members of this new family are: infectious pancreatic necrosis virus (IPNV) of fish, infectious bursal disease virus (IBDV) of chickens and Drosophila X virus (DXV) of fruit flies. Genome length mRNAs (lacking 3' poly A tails) are transcribed from the two genome segments by the virion-associated RNA polymerase using an assymmetric, strand-displacement mechanism. These are then translated to yield four polypeptides, all of which are found in mature virions. Genome segment B encodes VP1 (~90K) the putative RNA polymerase, whereas genome segment A encodes preVP2 (~52K) VP3 (~28K) and NS (~25K) polypeptides which are produced by co-translational cleavage of a ~105K polyprotein precursor (NH_2-preVP2-NS-VP3-COOH). The NS protein is an autocatalytic protease that cleaves the polyprotein at two places to generate the three polypeptides. During virus maturation, preVP2 (the major capsid polypeptide precursor) and NS are further trimmed at their carboxy terminal to yield VP2 and NSc. Sequence comparison between IPNV and IBDV genome segment A showed considerable sequence homologies in the preVP2 and VP3 regions, but no homology whatsoever in the region that encodes the NS protease.

S26.1

PHAGE EVOLUTION. A. Campbell, Department of Biological Sciences, Stanford University, Stanford, CA 94305, U.S.A.

There is general agreement that (1) the genomes of large DNA viruses originated from nonviral sources, such as chromosomes, plasmids, or transposons; (2) large viral genomes generally arose not from a single source but rather from compounding several separately derived gene blocks. There is less agreement on the extent to which recruitment of extraneous genes is ongoing in established viruses.

Evidence suggests that, in phage λ, current recruitment is rare. By contrast evidence for frequent homologous recombination in the wild is compelling. Therefore, the λ-related phages collectively may be regarded as a biological species; i.e., a population of individuals drawn from a common gene pool which is largely isolated from other gene pools, including that of the host. The components of the gene pool include the λ-related phages and also the defective prophages that clutter bacterial chromosomes.

Pairwise comparisons of DNA sequences of λ-related phages bear out the inference from early heteroduplex studies: Segments of good homology alternate with segments lacking detectable homology. Transitions from homology to non-homology are abrupt and frequently lie at boundaries of functional genomic domains; an observation for which no specific explanation is favored. The extent of sequence divergence, with conservation of map order suggests that λ is a mature species whose common ancestor was as genetically complex as its descendants.

S26.2

METHODS IN PHAGE ECOLOGY.

Sagar M. Goyal. Department of Veterinary Diagnostic Investigation, University of Minnesota, St. Paul, MN, U.S.A.

To study phage ecology, it is necessary to determine the distribution, fate, and survival of phage in the environment. This can only be accomplished if sensitive methods are available for isolation, enumeration, and identification of phage from diverse environments. Some of the methods that have been used for this purpose include: flocculation, ultracentrifugation, filter adsorption-elution, and enrichment techniques. An ideal method should be rapid, inexpensive, suitable for field use, and should result in good recoveries of a wide range of bacteriophage. In addition, it should be adaptable for use with large volumes of water and wastewater. To concentrate and detect small numbers of phage from large volumes of water, the virus adsorption-elution (viradel) procedure utilizing microporous filters seems to be preferred by most investigators. Subsequent to concentration and/or enrichment, the phage can be enumerated and identified by electron microscopy, phage serology, host range studies, and other phage characteristics. Advantages and disadvantages of these procedures will be discussed.

S26.3

CHARACTERIZATION OF PHAGES FROM INDUSTRIAL FERMENTATIONS

M. Teuber, Institute of Microbiology, Federal Dairy Research Centre, D-2300 Kiel, Federal Republic of Germany.

Bacteriophages have been described for all industrial fermentations using bacteria, e.g. production of antibiotics by Streptomyces and Bacillus spec. or of fermented food by lactic acid bacteria. In the case of high price compounds like antibiotics complete protection of fermentations by absolutely sterile conditions is economically feasible. However, in many food fermentations sterile fermentations are impossible for several reasons. As a consequence, bacteriophage problems are common and increasing with increasing fermentation volumes.

A classical food fermentation which is performed under absolutely unsterile conditions including equipment, substrate and aeration is the oxidation of ethanol to acetic acid in high acid, submerged vinegar fermenters. Fermentation slow downs are frequently encountered, however, only recently bacteriophages have been shown to be responsible for such events (Teuber et al. Biotechnol. Letters 9 37-38, 1987). Since the Acetobacter strains responsible for acetic acid production can usually not be isolated and propagated on semisolid media, a normal plaque test is not available for these phages. However, electron microscopy allows a qualitative and semiquantitative characterization of the phage level and status in vinegar fermenters. In addition, phages can be harvested from phage infected fermenters and be analyzed by classical biochemical methods.

S26.4

ECOLOGY OF LACTIC STREPTOCOCCAL PHAGES

A.W. Jarvis

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Lactic streptococcal phages isolated from cheese wheys were studied in order to elucidate the sources of phages in cheese plants. Phage DNAs were compared with one another by Southern-blot DNA-DNA hybridization, by electron microscopic heteroduplex experiments, and by restriction endonuclease cleavage analysis. DNAs from three phages belonging to the same morphological group but differing in host range were 67-94% homologous. Four different phages (by morphology and DNA-DNA hybridization) were isolated on one starter strain. Three of these were related by DNA studies to phages of similar morphology isolated previously on other starter strains. Examination of lactic streptococci from raw milk showed some strains could propagate lytic phages isolated from cheese wheys. It was concluded that (a) phages currently found in cheese plants have originated from only a few phage types; (b) 'new' phages have commonly developed from other lytic phages and (c) lactic streptococci in raw milk may act as propagating strains for phages and therefore contribute to the reservoir of phage in the plant environment.

S26.5

SEX PILUS PHAGES — VARIATIONS ON A VERY OLD THEME.

J.N. Coetzee, D. Bradley, R. Hedges. Department of Medical Microbiology, Faculty of Medicine, University of Pretoria, Pretoria, South Africa.

The first male (F^+, Hfr)-specific phages were described a quarter of a century ago. These were undulating filamentous ssDNA or RNA containing phages. The former adsorbed to tips of IncF-complex plasmid pili and the latter attached along entire shafts of these pili. Each of the types had distinct sensitivities to organic solvents.

This picture of sex pilus phages persisted up to 1981 since when phages adsorbing to pili of other plasmid Inc groups were described. Some of the new RNA phages adsorbed only to the distal shafts of pili, a couple were sensitive to chloroform and others were ts for replication. Lateral shaft-adsorbing filamentous phages were described which had the restricted host range of their RNA-containing counterparts. Another phage was curly in appearance while one was sensitive to ether. Thus many permutations of properties of the original phages were encountered. Finally a podophage was found to adsorb to shafts of pili encoded by a particular Inc group of plasmids.

S26.6

MYCOPLASMA VIRUSES: PHAGES WHICH INFECT WALL-LESS BACTERIA.

J. Maniloff, S.K. Poddar, G. Kampo, and C. Dascher. Department of Microbiology and Immunology, University of Rochester, Rochester, New York 14642, USA.

Unlike bacterial viruses that infect cells bounded by a cell wall, mycoplasma viruses have evolved to enter and propagate in mycoplasma cells bounded only by a single lipid-protein cell membrane. In addition, mycoplasmas have the smallest amount of genetic information of any known cells, so their complexity is constrained by a limited genetic coding capacity. As a consequence of these host cell differences, mycoplasma viruses have been found to have a variety of structures and replication strategies, different from those of the bacterial viruses.

Mycoplasma virus L2 is of particular interest. L2 is an enveloped virus containing circular, superhelical DNA of about 11.6 kbp. These temperate viruses are noncytocidal: following productive infection, with viral maturation by budding through the host cell membrane, lysogeny is established. Lysogenized cells are immune to superinfection, but can be induced, by ultraviolet light or mitomycin C, to produce L2.

Studies of the L2 genome are in progress. Two origins of replication have been mapped: most molecules replicate from both simultaneously. Sequencing of the L2 genome is almost complete, and the analysis of these data will be discussed.

S26.7
ARCHAEBACTERIAL VIRUS HOST SYSTEMS. Wolf-Dieter Reiter and Wolfram Zillig. Max-Planck-Institut für Biochemie, 8033 Martinsried, Federal Republic of Germany.

Viruses or virus-like particles have been found in all groups of archaebacteria. All known phages of halobacteria have a head-and-tail structure typical of many eubacteriophages. Most viruses of extremely thermophilic, sulfur-dependent archaebacteria, however, have an unusual morphology. The Thermoproteus tenax viruses TTV1, TTV2, TTV3 and TTV4 form rods with a complex substructure. The UV-inducible Sulfolobus virus-like particle SSV1 is lemon-shaped with small tail fibers attached to one end. SSV1 has been characterized in detail on the molecular level and its 15.5 kb circular DNA genome has been fully sequenced. The genes for three of its structural proteins have been identified. They are closely linked and co-transcribed. The comparative analysis of six transcriptional start points on the SSV1 genome showed that there is a highly conserved sequence around the transcriptional start and another highly conserved sequence 22-25 nucleotides 5' to it. Similar putative promoter sequences have been found in other archaebacteria suggesting an archaebacterial promoter consensus sequence.

S27.1

LEADER-PRIMED TRANSCRIPTION AND RNA RECOMBINATION OF CORONAVIRUS. Michael M.C. Lai, S. Makino, J.G. Keck, L. Soe, C.-K. Shieh, J. Fleming and S.A. Stohlman. Departments of Microbiology and Neurology, University of Southern California School of Medicine, Los Angeles, CA 90033, USA.

Mouse hepatitis virus (MHV), a coronavirus, utilizes a unique mechanism of leader-primed transcription to synthesize subgenomic mRNAs with a 70-nucleotide leader sequence. Evidence was obtained for the presence of free leader RNA species and for the possible role of an endonuclease activity in transcription. We have also shown the discontinuous nature of the transcription and replication of coronavirus RNA. The demonstration of these features of coronavirus RNA synthesis led to the discovery of high frequency of RNA recombination. By genetic crosses involving temperature-sensitive mutants, we have shown that recombinants constitute a significant proportion of progeny virus derived from mixed infection. Furthermore, some of the recombinants have multiple crossovers within the genome, confirming the high frequency of RNA recombination. We have further shown that RNA recombination could take place during intracerebral infection of mice. Thus, RNA recombination could be an important factor in virus evolution.

We have generated a series of RNA recombinants with genetic crosses within the gene encoding the peplomer proteins. Analysis of the genetic structure suggests that the neutralizing epitopes and determinants for viral neuropathogenicity are localized at the carboxyl terminal one-third of the peplomer protein of the virus.

S27.2

RIBOSOMAL FRAME-SHIFTING AS A TRANSLATIONAL CONTROL MECHANISM IN CORONAVIRUS IBV. Boursnell, M.E.G.[1], Brierley, I.[2], Binns, M.M.[1], Blok, V.[2], Bilimoria, B[2]. Brown, T.D.K.[2] & Inglis, S.C[2]. 1. Institute for Animal Disease Research, Houghton, UK. 2. Division of Virology, University of Cambridge, UK.

The complete nucleotide sequence of the coronavirus infectious bronchitis virus (IBV) has been determined from cDNA clones. The sequence of the 'unique' region of mRNA F, (the largest subgenomic RNA, which is thought to code for the RNA-dependent RNA polymerase), reveals the presence of two large overlapping open reading frames (ORFs) designated F1 and F2. The second or downstream ORF, F2, has no mRNA species associated with it, and analysis of the sequence at the F1/F2 junction suggests that ribosomal frame-shifting may be necessary for translation of F2.

In vitro transcription and translation systems have been used to examine the potential of the sequences at the F1/F2 junction for promoting frameshifting. Cloned IBV sequences and synthetic oligonucleotides have been inserted into cloned influenza virus genes in vectors suitable for transcription with SP6 RNA polymerase. Highly efficient ribosomal frameshifting can be demonstrated by analysis of the translation products on polyacrylamide gels.

S27.3

CHARACTERIZATION OF FUNCTIONAL DOMAINS OF THE E1-GLYCOPROTEIN OF CORONAVIRUS MHV A59. Heiner Niemann, Thomas Mayer, Matthias Falk, and Teruko Yamura. Institute of Medical Virology, Frankfurter Str. 107, D-6300 Giessen, FRG.

The E1-glycoprotein of MHV A59 (228 amino acids long) has three functional domains and determines the intracellular budding site of coronaviruses from perinuclear membranes. The hydrophilic N-terminal domain (28 amino acids) carries exclusively O-linked carbohydrates which exhibit in conjunction with the N-terminal amino acid sequence M-blood group activity. The inner part of E1 (amino acids 29 to 109) is hydrophobic with the exception of the short stretch from amino acids 44 to 50. The hydrophilic C-terminus interacts with the viral nucleocapsid and thus plays the role of a matrix protein during virus maturation.

Using site directed mutagenesis we have constructed various deletion mutants of E1. In addition, Ser(3) was mutated into an Asn residue to create an N-glycosylation site (Asn(3)-Thr-Thr). Several new restriction sites were introduced into the E1-gene, to study the role of the individual hydrophobic domains. The first membrane spanning domain (amino acids 29 to 43) serves as an SRP dependent signal for membrane integration as evidenced by in vitro transcription translation studies. A second signal sequence which has not yet been adequately localized, is present between Phe(51) and Phe(109). Microinjection of capped and polyadenylated mRNA into CV-1 cells indicated that the signal for transport of the E1-protein into perinuclear membranes is located on the E1-protein itself. The hydrophilic N-terminal and the C-terminal domains of E1 do not play a role in this transport. We have expressed the E1-gene and the mutants thereof from recombinant vaccinia virus vectors. The migration of the E1-peptides was studied by indirect immunofluorescence and by metabolic labelling using the O- and N-glycosylated carbonhyorate side chains as markers for the intracellular transport of E1.

S27.4

CONTROL OF CORONAVIRUS (CV) EXPRESSION IN THE CENTRAL NERVOUS SYSTEM (CNS). S. Dales. Department of Microbiology and Immunology, University of Western Ontario, London, Canada.

Replication of murine coronaviruses MHV, and JHMV in the CNS of rats and mice is controlled by multifactors including (a) T cell immune responses, (b) host age-related differentiation of neural cell targets, (c) host strain-related genetic specificity, (d) CV strain-related specificity. Selected examples of controls by mechanisms (a)-(d) will be reviewed briefly. More detailed information will be provided concerning item (b). Special attention will be given to the consequences arising from modulation of the adenylate cyclase and connected metabolism by the differentiation inducer cAMP and related compounds upon CV expression in primary explants of astrocytes and oligodendrocytes.

S27.5

IMMUNOPATHOLOGICAL ASPECTS OF CORONAVIRUS-INDUCED SUBACUTE DEMYELINATING ENCEPHALOMYELITIS IN LEWIS RATS. R. Dörries, S. Schwender, H. Wege, R. Watanabe, P.T. Massa, H. Harms and V. ter Meulen, Institut für Virologie und Immunbiologie der Universität, Würzburg, Fed. Rep. Germany.

The murine coronavirus JHM is known to cause a clinically relevant subacute demyelinating encephalomyelitis (SDE), when injected intracerebrally into Lewis rats.

A strong involvement of the immune system in the disease is indicated (1) by perivascular cuffing of T-cell lines specific for myelin basic protein in the CNS of healthy recipient animals; (2) by induction of a clinical relapse in rats which have recovered from SDE by JHM virus-specific T-cell lines; (3) by hyperinducibility of class II antigens on astrocytes and (4) by the lack of a significant intrathecal JHM-specific antibody response in SDE rats. Histological localization of lymphocytes, macrophages, glia cells, class II proteins and virus-specific proteins in serial cryostate sections of brain and spinal cord of SDE and non-diseased rats revealed complex topographical relations between CNS-resident immune cells and virus-infected brain cells.

The data suggest that in the absence of a protective, virus-specific antibody response T cell populations carrying either auto- or virus-specificity may determine largely the pathogenesis of JHM virus induced SDE.

S27.6

OPPORTUNISTIC PATHOGENICITY OF MURINE CORONAVIRUSES. K. Fujiwara, N. Goto, K. Midoro, N. Okada and K. Nakanaga. Department of Veterinary Pathology, Faculty of Agriculture, University of Tokyo, Tokyo 113, Japan.

Wild strains of murine hepatitis virus (MHV) are mostly opportunistic pathogens causing infantile diarrhea in sucklings or wasting syndrome in athymic nude mice while euthymic adult mice are inapparently infected. Nude mice exhibit no acute or fulminant hepatitis but a variety of progressive subacute or chronic disorders such as hepatitis, pancreatis, encephalitis, glomeruronephritis, etc. The subacute or chronic hepatitis in the immunodeficient hosts due to persistent and progressive infection with low or moderately cytopathic viruses might provide a useful model for the same type of disease in man.

After inoculation with a very low-virulence MHV, athymic nude mice died in 3 weeks to 3 months showing degeneration and necrosis of hepatocytes with persistently active inflammatory response and some fibrosis. Throughout the course of infection infective virus could be recovered and viral antigen as well as virions were detectable within hepatocytes. Most of infected animals were shown to have some MHV-reacting serum immunoglobulins but no virus neutralizing antibody. Such balanced state established between the opportunistic MHV and immunodeficient host animals has been unable to be produced in immunologically normal hosts infected with highly hepatitogenic laboratory strains of MHV.

S28.1

PAPILLOMA VIRUSES: ESTABLISHMENT OF LATENCY. M. Botchan, Dept. of Molecular Biology, University of California, Berkeley, CA, USA

ABSTRACT NOT AVAILABLE

S28.2

REGULATION OF AAV GENE EXPRESSION. B. Carter, E. Mendelson, J. Trempe, C. Lally, N. Chejanovsky, B. Redemann, I. Miller. NIDDK, NIH, Bethesda, Md. 20892, USA.

Adeno-associated virus (AAV) promoters (p5, p19, p40) give overlapping mRNAs. p40 mRNAs code for capsid proteins. p5 and p19 mRNAs code for rep gene proteins: rep78 and rep68 from p5 RNA or rep52 and rep40 from p19 RNA. Rep mediates three trans-acting functions: DNA replication and negative and positive control of the p40 transcription unit. Positive control increases cytoplasmic RNA levels but does not appear to require sequences in cis immediately upstream of the p40 promoter. Negative control appears to decrease translation and occurs for mRNAs from other promoters in addition to p40. Negative regulation is predominant in some (293) but not other (Hela) human cell lines. The coding region for rep52 and rep40 is completely embedded within that for rep78 and rep68. Thus it is difficult to determine independent roles of each rep protein in transient expression assays. However p5 rep proteins are required but the additional carboxyl terminus regions are dispensible for all three functions. AAV vectors integrated into cellular chromosomal sites appear to discriminate a property of p19 rep proteins in the absence of p5 rep proteins. Rep proteins appear in the cell nucleus by immunofluoresence but cell fractionation readily extracts rep52 and rep40 into the cytoplasm fraction. Rep78 and rep 68 appear to be more tightly bound in the nucleus.

S28.3

EARLY TRANSCRIPTION REGULATION IN HERPESVIRUSES. B. Fleckenstein, Institut für Klinische und Molekulare Virologie der Universität Erlangen-Nürnberg, D-8520 Erlangen, W.-Germany.

Herpesvirus gene expression follows, in general, a cascade of three regulated phases (immediate early = IE, early, late) of gene expression during productive infection. The herpesgroup is highly heterogeneous, however, with regard to the modes of transcriptional regulation. The IE-genes of herpes simplex virus, a prototype human alphaherpesvirus, are activated in trans by a structural virion component. IE-genes of human cytomegalovirus (HMCV), a member of the beta-subgroup, are under the control of a very strong constitutive enhancer; it is organized in a modular fashion containing multiple sets of short repetitive elements; each repeat is a sequence-specific protein binding site and was identified as a functional enhancer element. A transcription enhancing sequence which is able to substitute for the SV40 enhancer was also found in a gammaherpesvirus, the lymphotropic H. saimiri; it seems unrelated in functional organisation with the HCMV IE-enhancer. Although structural protein genes of various herpesviruses are often well conserved in evolution, many examples show that regulatory proteins and promoter/enhancer-sequences are highly divers, even in closely related herpesviruses.

S28.4

ANALYSIS OF ENHANCERS OF POLYOMAVIRUS MUTANTS ISOLATED FROM EMBRYONIC CELL LINES. M.Satake, K.Furukawa, Y.Yamaguchi & Y.Ito. Institute for Virus Research, Kyoto University, Sakyo-Ku, Kyoto 606, Japan

We have been studying mutant enhancers in relation to interaction with factors specific to stages of differentiation. An F9 mutant, F441 (Fujimura et al, Cell,23,809,1981) carries a single base change at nt 5233. Enhancers of most of the F9 mutants have a duplicated segment of viral DNA encompassing the nt 5233. We prepared oligonucleotides spanning from nt 5218 to 5339 of WT and F441 and examined their biological activity by a transient CAT assay in F9 cells. The F441 oligonucleotide stimulated expression of CAT gene in cis in F9 cells when linked at an upstream position at both orientation. When dimerized, several fold higher activity was observed. The results suggested that the point mutation makes the segment a binding site for a positively functioning factor(s), rather than to eliminate a binding site for a negatively regulating factor(s). Direct demonstration of factor binding is also being done.

S28.5

MAPPING FUNCTIONAL DOMAINS ON SV40 LARGE T ANTIGEN. M. J. FORD, S. MOLE, J. GANNON, G. GOUGH, & D. P. LANE. Imperial Cancer Research Fund, Clare Hall Laboratories, South Mimms, Herts, EN6 3LD, England.

The large T antigen of SV40 virus plays a key role in both the lytic and transforming activities of the virus. This 708 amino acid phosphoprotein shows a wide spectrum of biological activies in vivo and a number of discrete biological activities in vitro. The purified protein will bind specifically to DNA sequences at the viral origin of replication. It displays an ATP-dependent helicase activity and will initiate DNA synthesis in vitro in the presence of crude cell extracts. As well as being associated with the oncogene product p53, we have recently shown that T antigen is associated with DNA polymeraseα. By using these different in vitro assays, a panel of inhibiting monoclonal antibodies and biologically active prokaryotic fusion proteins we have been able to map functional domains on large T antigen.

Because of T antigen's specific interactions with evolutionary conserved host proteins (p53 & polymerase α) we are actively looking for the putative cellular homologues of T. We have identified, cloned and partially sequenced a nuclear host protein that shares homology with large T antigen. This protein, termed p68, is growth regulated and evolutionary conserved in vertebrates. p68 is recognized by an anti-T monoclonal antibody, PAb204, that can inhibit the ATPase and helicase activies of T antigen and its ability to initiate DNA replication. From the area of sequence homology and the use of prokaryotic fusion proteins and synthetic peptides we have mapped the epitope to less than 16 amino acids on each protein.

S28.6

FUNCTION AND REGULATION OF AUTONOMOUS PARVOVIRAL NON-STRUCTURAL (NS) PROTEINS

Peter Tattersall, Robyn Moir, Susan Cotmore & Edith Gardiner; Departments of Laboratory Medicine and Human Genetics, Yale School of Medicine, New Haven, Connecticut, USA.

Fibrotropic and lymphotropic strains of minute virus of mice (MVM) are reciprocally restricted for the initiation of viral transcription in each other's normal host cell. The region responsible for the ability of the fibrotropic strain to turn on its genes in fibroblasts, maps to a small hypervariable region of the capsid gene. Extended host range mutations also map to this small region, and involve changes in the primary sequence of the coat protein. Comparison natural infection with plasmid-mediated transfection shows that the proper establishment of transcription patterns is particle-mediated, and that productive infection of differentiated cells is controlled at this level also. During infection of a productive host, trans-activation of expression from the P38 promoter occurs. Site-directed mutagenesis of NS-1 and NS-2 coding sequences shows that both of these products of P4 transcription are required for trans-activation of P38-directed expression. Trans-activating activity is not expressed during restrictive infection, and expression from either promoter of a virion-delivered, but restricted, genome cannot be rescued in trans by expression from a co-infecting, non-restricted virus. These results suggest a requirement for interaction between the incoming virion and a differentiation-specific intra-nuclear "receptor", for the establishment of active viral transcription templates, both during the initiation of infection and later, during the trans-activation phase.

S29.1

LONG-DISTANCE TRANSPORT OF ARTHROPOD VECTORS: CONCEPTS AND APPROACHES. M. E. Irwin, University of Illinois and Illinois Natural History Survey, 607 E. Peabody, Champaign, IL 61820, U.S.A.

Many plant viruses depend on winged arthropods for movement from host to host, the portion of the epidemiological cycle that directly regulates virus movement; other important portions govern probabilities of and rates for epidemics. Yet, little is known about how vectors move or how to study this movement. We developed conceptual frameworks and methodologies to investigate vector movement, both vertical displacement and horizontal translocation. Wind transport was studied using an objective trajectory model; flight energy utilization provides flight duration information. Together these techniques give good source area resolution, especially when coupled with allozyme studies. Vertical distribution of vectors is obtained by helicopter-mounted aerial collectors. Although focused on aphids as vectors of plant viruses, the concepts and approaches are generally applicable to other plant and animal virus vectors.

S29.2

COEVOLUTION OF INSECTS AND PLANT VIRUSES. L. R. Nault, Department of Entomology, The Ohio State University, Wooster, OH 44691, USA.

The Auchenorryncha transmit 38 plant viruses from among six distinct virus groups: reoviruses, rhabdoviruses, rice stripe virus group, maize rayado fino virus (MRFV) group, geminiviruses and the maize chlorotic dwarf virus (MCDV) group. Viruses from the first three groups multiply in their vectors, are transovarially transmitted and are postulated to have originated as insect viruses that secondarily adapted to plant hosts. The MRFV group replicate in their vectors, but are not transovarially transmitted; it is uncertain if these viruses originated as plant viruses or as insect viruses. Geminiviruses are circulative and neither replicate in their vectors nor are transovarially transmitted. The MCDV group are stylet/foregut borne and neither multiply nor are transovarially transmitted in their vectors. Viruses in these latter two groups likely originated in plants as seed-borne viruses that later lost this property after they evolved relationships with insect vectors. Insects from more than one homopteran family can transmit reoviruses, rhabdoviruses and geminiviruses although no virus is transmitted by vectors from more than one family. These distributions among vector families are best explained by association by descent of viruses with vectors for reoviruses and geminiviruses, and by colonization (horizontal transfer) of viruses from one homopteran family to another via a common host plant for rhabdoviruses.

S29.3

IMPACT OF NEW TECHNOLOGY ON STUDIES OF VIRUS EPIDEMIOLOGY. J.W. Randles. Department of Plant Pathology, Waite Agricultural Research Institute, University of Adelaide, S. Australia.

'New technology' may be defined as that which provides improved speed, sensitivity and specificity to the detection of viroids, viruses and virus strains. Methods in use and under development depend upon the detection of virus-specific protein and nucleic acid. 'Speed' is generally best achieved by serological methods, 'sensitivity' relies upon amplification of antibodies or complementary nucleic acids by labelling with radioactive isotope or enzymes, and high 'specificity' can be achieved with epitope specific monoclonal antibodies and sequence specific probes. Less stringent group specific probes can be devised to detect general physical properties such as shape or structure.

Studies of epidemics require the identification of pathogens, their sources, their distribution and their vectors. Examples will be presented in which (i) gel electropheretic methods and nucleic acid probes are used to evaluate the epidemiology of the coconut cadang-cadang viroid (ii) nucleic acid hybridization shows the geographical distribution of one strain of bean yellow mosaic virus in relation to others, and (iii) DNA cloning, blotting and monoclonal antibody have been used to describe the possible physical properties of the presumed viral agent of foliar decay disease.

S29.4

THE EMERGING ECOLOGY OF HANTAAN AND RELATED VIRUSES, THE CAUSE OF HEMORRHAGIC FEVER WITH RENAL SYNDROME (HFRS). Zhi-Yi Xu. Department of Epidemiology, Shanghai Medical University, Shanghai, China.

In China, Hantaan virus has been isolated from hare, shrew, cat and weasel and transmission of the virus from cat to human has been confirmed. Thus, the host range in Hantaanvirus group is wider than previously thought. The virus which canbe transmitted with rodent excretions were detected in Gamasoid mites(Hemolaelaps glasgowi) and the transovarial transmission of the virus has been demonstrated. Being not anthropophilic, the mite may transmit the virus among rodents.

In China, human cases were associated mainly with Apodemus agrarius or Rattus norvegicus. Rat associated disease is milder. But the antigenic differnces between the two related viruses have not been well defined. The Apodemus associated HFRS outbreaks were observed in semicultivated swampy land and valleys while the rat associated diseases occurred in both urban and rural areas. The marked rise in HFRS incidence recently in China maybe explained by the increase of rodent population in dwellings due to store of grains in living houses of farmers in recent years. Proportion of rat associated cases increased. The change is under study.

S29.5

VIRUSES IN SEARCH OF VECTORS. T.P. Monath, Division of Vector-Borne Viral Diseases, Centers for Infectious Diseases, Centers for Disease Control, Fort Collins, Colorado, U.S.A.

Vesicular stomatitis is an economically important disease of cattle, horses, and pigs in the Americas. Despite years of study, many aspects of the biology, epidemiology, and pathogenesis of this disease remain ill-defined, in particular the modes of virus transmission and maintenance in nature. Insects have been suspected to play a role in biological transmission because of the summertime distribution of disease, the movement of outbreaks in valleys and wet pasturelands, the occasional virus isolation from arthropods, and experimental evidence for arthropod infection. Nevertheless, doubt remains about the vector role of arthropods since vertebrate hosts develop only evanescent, low-titered viremias and since parenteral inoculation results in inapparent infection without vesicular lesions. Recent data regarding the role of insects in vesicular stomatitis virus ecology will be presented and alternative modes of transmission discussed.

S30.1

PENETRATION OF RABIES VIRUS AND ITS AVIRULENT DERIVATIVE IN THE PERIPHERAL NERVOUS SYSTEM OF THE MOUSE. A. Flamand, C. Derbin, P. Kucera* and M. Dolivo*. Laboratoire de Génétique des Virus, CNRS, Gif sur Yvette, France and *Institute of Physiology, University of Lausanne, Switzerland.

CVS rabies virus or its avirulent mutant (substitution in the viral glycoprotein of arginine 333 by glutamine) were inoculated into the triceps surae muscle of adult mice. The infected cells were searched for in serial sections of the muscle, dorsal root ganglia and spinal cord stained with fluorescent antiviral nucleocapsid antibodies.

In the case of CVS, no viral fluorescence could be detected one day after inoculation. After two days, no fluorescence was present in the muscle but, simultaneously, few sensory and motor neurons in the ganglia and ventral horn of the lumbar cord were already brightly fluorescent.

In mice inoculated with avirulent strain, only the ganglia were infected after two days indicating a restricted capacity of the mutant to penetrate the motoneuronal endings.

These results complete our previous study of penetration of these two strains after intraocular inoculation and confirm that this single mutation dramatically changes the affinity of the virus for various kinds of nerve endings (J. Virol. 55: 158-162, 1985).

S30.2

EFFECTS OF MUTATIONS IN THE CYTOPLASMIC DOMAIN ON TRANSPORT AND OLIGOMERIZATION OF THE VESICULAR STOMATITIS VIRUS GLYCOPROTEIN. John Rose, Jun-Lin Guan, Robert Doms, Ari Helenius, and Aino Ruusala. Departments of Pathology and Cell Biology, Yale University School of Medicine, New Haven, Ct, 06510, U.S.A.

Previous studies from our laboratory have shown that deletion or substitution mutations affecting the amino acid sequence in the cytoplasmic domain of the vesicular stomatitis virus (VSV) glycoprotein (G) can reduce the rate of G protein transport from the endoplasmic reticulum (ER) to the Golgi apparatus. To determine if these effects on transport might be due to indirect effects on oligomerization of G protein, we analyzed the kinetics of oligomerization for several of these altered G proteins. The results show that there is no correlation between transport and oligomerization of these mutant proteins. Analysis of six mutants slowed or blocked in their transport from the ER showed that all formed trimeric G protein with a half-time of under 10 minutes, at least as fast as the wild-type protein. These studies and other studies on membrane anchored forms of secretory proteins suggest that the effects of changes in the cytoplasmic domain of G protein are independent of effects on the ectoplasmic domain.

S30.3

THE VSV POLYMERASE: CLONING AND FUNCTIONAL EXPRESSION. M. Schubert. Laboratory of Molecular Genetics, NINCDS, National Institutes of Health, Bethesda, Maryland, U.S.A.

The 6.4 kb gene encoding the multifunctional 241 kDa polymerase protein L of Vesicular stomatitis virus (VSV) was assembled from five partial, overlapping cDNA clones. The L gene construct has the consensus sequence derived from three cDNAs. The recombinant L protein was functionally expressed in COS cells and complemented ts L gene mutants at the nonpermissive temperature. Expression of low levels of L protein is optimal for the complementation of the mutant. Surprisingly, expression of high levels of L protein does not complement the mutant, but arrests the mutant and even wild-type virus. The arrest is caused by high levels of polymerase prozein L itself and not by the amplification of the vector and/or other vector products. These data suggest that a fine balance between viral proteins and/or the viral L protein and host factors is necessary for virus replication.

To map the sites of interactions between the VSV polymerase subunits L and NS and the template, recombinant NS deletion mutants were constructed and transcribed in vitro using T7 RNA polymerase. These mutant proteins, translated in vitro using reticulocyte lysates, were bound to purified L, ribonucleocapsid and recombinant N protein and stable complexes were immune co-precipitated using anti-L and anti-N antibodies. These immune co-precipitations revealed that the NS protein is a bivalent protein with two separate binding domains. The highly phosphorylated and negatively charged amino half contains the L binding domain, the carboxy half contains the N/nucleocapsid binding domain.

S30.4

PHOSPHOPROTEINS OF VESICULOVIRUSES. L.A. Prevec, Dept. of Biology, McMaster University, Hamilton, ON, Canada

ABSTRACT NOT AVAILABLE

S30.5

USE OF MONOCLONAL ANTIBODIES TO DEFINE THE FUNCTION OF VSV NS PROTEIN. S. Emerson, M. Williams. University of Virginia Medical School, Charlottesville, Virginia, USA.

Fourteen monoclonal antibodies to the NS phosphoprotein of vesicular stomatitis virus were isolated after inoculation of mice with purified, native NS protein. Epitope mapping was done by competition binding and immune precipitation of in vitro synthesized deletion mutant proteins. Most of the antibodies were raised against the carboxyterminal half of the NS protein rather than the highly charged, phosphorylated amino terminal half. Three of the antibodies were tested for their effect on transcription. Antibody 4F11 bound to NS protein on the template but had no effect on transcription. Antibody 6D11 inhibited transcription of leader RNA, mRNAs and the AC initiation dinucleotide. Antibody 2A2 inhibited mRNA synthesis but did not prevent leader RNA nor AC synthesis. The 2A2 antibody also strongly cross reacts with the viral nucleocapsid protein and with a cellular filament protein.

S30.6

THE VSV MATRIX PROTEIN: STRUCTURE-FUNCTION RELATIONSHIPS. Robert R. Wagner, Joshua B. Shipley, Ranajit Pal and Yan Li. Department of Microbiology, University of Virginia, Charlottesville, Virginia, U.S.A.

We are mapping the sites on the VSV matrix (M) protein that recognize and bind to the membrane and to the ribonucleoprotein core, and in so doing inhibit viral transcription. Monoclonal antibody to one antigenic determinant (epitope 1) reverses inhibition of transcription by wild-type M protein but fails to bind to the M protein of mutant *tsO23*(III). We have mapped epitope 1 of *wt* M protein to amino acids 17-31 by cleavage with trypsin and N-chlorosuccinimide, as well as by testing synthetic oligopeptides. Mutant *tsO23*(III) M protein has a Gly→Glu substitution at amino acid 21 which results in loss of epitope 1 and transcription inhibition. Computer modeling (in collaboration with Matthew Pincus) by energy minimization reveals that dodecapeptide Lys15-Pro26 markedly alters its three-dimensional structure following Gly→Glu21 substitution. We are also producing site-directed mutations in this region of M-gene expression vectors to determine the linear and three-dimensional structure of M protein that controls biological function and exposure of antigenic determinants.

S31.1

AUTOCATALYTIC MATURATION CLEAVAGE IN PROVIRIONS OF A SMALL ICOSAHEDRAL INSECT VIRUS. T. M. Gallagher and R. R. Rueckert. Biophysics Laboratory, University of Wisconsin, Madison, WI, USA.

The spherical shell of Flock House virus (FHV), a member of the insect virus family Nodaviridae, is composed of equimolar amounts of two mature proteins, beta (363 AA) and gamma (44 AA), plus minor amounts (<20%) of a precursor protein alpha (407 AA). Nucleic acid and amino terminal protein sequencing showed that beta and gamma were generated by cleavage of the alpha chain between Asn 363 and Ala 364.

Radiolabelled "provirion" (PV) particles, obtained by pulse-labelling infected Drosophila cells for an hour with ^{35}S-methionine, were very rich in alpha chains (>95%). In vitro incubation of PV yielded mature virions containing primarily beta and gamma. Alpha cleavage followed first order kinetics, with a half life of 3.6 hours. Cleavage was not affected by a broad spectrum of protease inhibitors, nor when PV were rapidly purified away from cytoplasmic extracts by either immune precipitation or sucrose gradient sedimentation. Unlike mature virions, PV were unstable in SDS; dissociated alpha chains did not cleave. We conclude that cleavage is an autocatalytic process, leading to virion stability.

This proteolysis of alpha to beta and gamma, which may be analogous to the picornaviral VP0 cleavage, represents the first reproducible in vitro system for the study of maturation cleavage.

S31.2

NUDAURALIA VIRUS. M.V. Nonur, Dept. of Biological Sciences, Purdue University, West Lafayette, IN, USA

ABSTRACT NOT AVAILABLE

S31.3

THE MOLECULAR BIOLOGY OF CRICKET PARALYSIS VIRUS. L.King, N.Moore. Department of Biological Sciences, Oxford Polytechnic, Oxford, England.

Cricket paralysis virus is an insect picornavirus which has three major capsid proteins with molecular weights of 30-35 kD. In infected Drosophila melanogaster cells, protease inhibitors and amino acid analogues inhibit protein processing and the presence of a polyprotein can be demonstrated. Pactamycin mapping gives the order of the structural proteins on the RNA.

The single stranded RNA is polyadenylated and has a covalently linked protein. DNA complimentary to the viral RNA has been cloned and sequenced.

S31.4

Polydnaviruses: Multipartite DNA Viruses. J.G.W. Fleming, Dept. Entomology, Texas A&M University, College Station, TX 77843, USA.

In several species of braconid and ichneumonid wasps polydnaviruses replicate in the calyx oviduct cells. At oviposition female wasps inject virus together with their eggs into lepidopteran host larvae where viral genes are expressed throughout the course of wasp endoparasitic development. Polydnaviruses induce several dramatic physiological changes in the parasitized host, including altered hormone titers and hemocyte profiles. Experiments with washed wasp eggs and polydnaviruses indicate that host hemocytes do not encapsulate wasp eggs if virus is present, suggesting that polydnaviruses compromise host cellular immune responses in a manner dependent on viral gene expression and thereby permit wasp endoparasitic development.

Polydnaviruses have genomes composed of multiple superhelical dsDNAs (SH DNAs). Although the >28 SH DNAs in the genome of the Campoletis sonorensis virus (CsV), the best characterized polydnavirus, have a significant amount of sequence homology, blot hybridization studies indicate that the SH DNAs are not identical. Studies using probes homologous to viral mRNAs found in parasitized hosts indicate that selected viral genes may be present on several but not all of the viral SH DNAs, indicating that the genome is transcriptionally and physically multipartite. Two related genes with partial sequence homology have been detected on SH DNA W, suggesting that gene families may exist in the CsV genome. A family of 540 bp repeated elements which are present in one or more copies on the majority of the viral SH DNAs has been identified. These data suggest that recombination between SH DNAs may have occurred. DNA from wasp tissues which do not contain significant amounts of virus appears to contain a differently organized form of CsV DNA which suggests that the CsV DNA(s) are integrated. Possible viral transmission mechanisms in the wasps are discussed.

S31.5

ORGANIZATION AND REGULATION OF BACULOVIRUS GENES. L.K. Miller, Dept. of Entomology, University of Georgia, Athens, GA, USA

ABSTRACT NOT AVAILABLE

S31.6

BACULOVIRUSES AS GENE EXPRESSION VECTORS.

M.A. Cochran, B.L. Ericson, J.D. Knell and G.E. Smith. Department of Molecular Biology, MicroGeneSys, Inc., West Haven, Connecticut, USA.

The baculovirus-insect cell vector system has been used as a high-efficiency eukaryotic cloning and expression method for the production of recombinant proteins in cultured insect cells. This system can be applied to two general areas: the production of recombinant protein for diagnostic, therapeutic or vaccine applications; and, the construction of recombinant baculoviruses for use as bioinsecticides.

We will present data on the use of baculovirus recombinants for the production of subunit vaccines and on the design of recombinant baculoviruses for use as bioinsecticides.

S32.1

EVOLUTIONARY SOURCES OF VIRAL GENES.

Adrian Gibbs, Research School of Biological Sciences, Australian National University, Canberra, 2601, AUSTRALIA.

Most known viruses fall into one or another of 100-200 groups)'genera'). Each of these are probably the descendants of a single ancestral virus. The members of each group usually have closely similar biochemical and ecological life-cycles, but often differ in their host ranges, virulence and vector species. Recent studies have shown some examples of unequivocal homologies between members of different groups either in the sequences of their genomes or in the structures of their coat proteins.

Thus most extant viruses, it seems, come from a small genetic base; they arose as various combinations of a few genes, and have 'speciated' as they co-evolved with their hosts or have adapted to new ones, however their ability to 'colonize' taxonomically distant hosts seems to be limited.

S32.2

RETROVIRAL EVOLUTION AND THE FIDELITY OF GENOMIC REPLICATION. **B.D. Preston[1], J.D. Roberts[2], T.A. Kunkel[2] and L.A. Loeb[1]. [1]Department of Pathology, University of Washington, Seattle, WA, USA and [2]Laboratory of Genetics, National Institute of Environmental Health Sciences, Research Triangle Park, NC, USA.**

The sequence heterogeneity and high mutation rates of retroviruses suggest that retroviral replication is an error-prone process. This hypermutability can be duplicated in vitro by copying DNA templates with purified reverse transcriptases (RTase). Analyses of RTases in a ϕX174 am3 reversion mutation assay (Weymouth & Loeb, PNAS 75:1924, 1978) show that mutations are produced during in vitro DNA synthesis at a rate of approximately 1/500 to 1/15,000. In contrast, a multisubunit complex of DNA polymerase-α, the host polymerase responsible for cellular and proviral DNA replication, exhibits an error rate of only 1/500,000. Comparison of RTase and polymerase-α in an M13 lacZα forward mutation assay (Kunkel, JBC 260:5787, 1985) also shows that these polymerases are distinguishable by the distribution and type of mutations produced. DNA synthesis by Avian Myeloblastosis Virus RTase exhibits three unique characteristics: 1) low fidelity; 2) preferential catalysis of transversion mutations; and 3) stabilization/utilization of misaligned primer-templates. These studies provide a mechanistic explanation for the high mutation rates of retroviruses based on the infidelity of reverse transcriptases.

S32.3

FAST EVOLUTION IN THE GENES OF HUMAN INFLUENZA A VIRUSES; SLOW EVOLUTION IN INFLUENZA B AND C VIRUS GENES. **P. Palese, M. Yamashita and M. Krystal. Dept. of Microbiology, Mount Sinai School of Medicine, New York, N.Y. 10029.**

Sequence analysis of the NS genes of more than 15 human influenza A virus strains isolated between 1933 and 1985 revealed a steady accumulation of mutations over time. These changes suggest an average evolutionary rate of 2×10^{-3} substitutions/site/year for this gene. The finding also suggests that influenza A viruses in general show one major (dominant) evolutionary lineage. In contrast, sequence analysis of the NS and the HA genes of influenza B and C viruses isolated over a period of approximately 40 years show a different evolutionary pattern. At any time, influenza B and C viruses deriving from different evolutionary lineages cocirculate in the human population. Depending on the strains used for analysis, few changes may be seen in genes of influenza B and C viruses which were isolated many years apart. This suggests that the evolutionary rate in influenza B and C virus genes is much slower than in human influenza A virus genes. We now plan to measure the mutation rates for influenza B and C viruses, compare them with the rate for influenza A viruses, and attempt to correlate evolutionary rates of viruses circulating in nature with their mutation rates established in tissue culture systems.

S32.4

TWO ENTEROVIRUSES CAUSING ACUTE HAEMORRHAGIC CONJUNCTIVITIS -- ENTEROVIRUS 70 AND COXSACKIE A24 VARIANT (EH 24 TYPE)

N.Takeda, K.Miyamura, M.Tanimura, and S.Yamazaki. National Institute of Health, Tokyo, Japan.

In an attempt to elucidate the origin and evolution of the enteroviruses in nature, the genome RNAs of EV70 and CA24 isolates, which were obtained from different parts of the world over the past 15 years, were subjected to RNase T1-resistant oligonucleotide mapping analysis and phylogenetic trees were constructed. Base sequence variation among isolates was deduced from the number of base difference estimated from pairwise comparison of the oligonuleotide spots and used as a genetic distance between isolates. The base substitution rate of RNA genome of EV70 was estimated to be 1.8×10^{-3}/base/year from regression analysis of the genetic distance of the isolates against the time of isolation. Based on the substitution rate, genetic distance and sampling time of the isolates, the phylogenetic tree was constructed using UPGMA. The tree of EV70 was in good agreement with epidemiological evidence of this virus and supported our previous hypothesis that evolution of EV70 started from a single common ancestor. The time of its emergence was estimated to be 1967 ±15 month. The virus evolved in a divergent fashion yielding genetically polymorphic viruses in the world. We also applied the same method to CA24 variant, which has been known as another etiological agent of AHC but genetically unrelated with EV70. The tree indicated that the virus evolved in a similar fashion with nearly the same substitution rate.

S32.5

DYNAMICS OF POLIOVIRUS VARIATION DURING NATURAL REPLICATION. **Olen Kew, Baldev Nottay, Rebeca Rico-Hesse, Mark Pallansch. Centers for Disease Control, Atlanta, GA 30333, USA.**

Rapid genomic variation of vaccine-derived and wild polioviruses during replication in humans occurs by both selection of new mutants (base substitutions, deletions) and recombination. Mutational variants and recombinants of the poliovaccines, selectively amplified during intestinal replication, were characterized by primer-extension sequencing. Recombinant genomes frequently contained sequences derived from all three vaccine strains. Probe hybridization and sequence characterization of isolates plaqued directly from excreted virus demonstrated the concurrent infection of some vaccinees with independently produced recombinants of the same serotype. Analyses of wild poliovirus isolates (all three serotypes) by partial genomic sequencing allowed us to: (1) determine the patterns of nucleotide sequence divergence among isolates from different regions of the world, (2) identify numerous geographic foci of independent endemic circulation of wild polioviruses, (3) establish previously unsuspected epidemiologic links between outbreaks in distant communities, (4) monitor the displacement from the environment of pre-existing genotypes by extrinsic genotypes, (5) recognize the recombinant origins (vaccine-wild; wild-wild) of some epidemic polioviruses, and (6) assess the possible vaccine derivation of some endemic case isolates having unusual genomic sequences.

S32.6

EXTENSIVE HETEROGENEITY OF HIV GENOMES. W. Parks*, E. Parks*, M. Staag**, J. Gibbons**, F. Wong-Staal***, G. Shaw**, B. Hahn**. *University of Miami, Miami, FL, ** University of Alabama at Birmingham, Birmingham, AL, and ***Laboratory of Tumor Cell Biology, NCI, Bethesda, MD.

The AIDS virus, HIV, has been shown to exhibit striking genomic variation when isolates obtained from different individuals are compared. To assess the extent of genetic variation present in vivo we isolated HIV from peripheral blood mononuclear cells of two infected patients (RJS and MPF), generated large recombinant lambda phage libraries and identified and characterized multiple clones of HIV to search for evidence of genomic diversity in vivo. Full length viral clones from 2 libraries were obtained and analyzed by restriction endonuclease mapping. Out of 30 RJS clones, 13 highly related yet genetically distinct genotypes were evident. Of the 16 MPF clones, 9 highly related yet genetically distinct genotypes were apparent. Certain genotypes were represented by more than one clone and these, in turn were evident as predominant species on the original Southern blot patterns of infected cellular DNA. HIV passaged in vitro by sequential limiting dilutions did not exhibit similar degrees of genomic variation. These data indicate that extensive genetic variation is generated in vivo and the degree of viral heterogeneity has been underestimated. Unless cloned, most isolates are actually complex mixtures.

S32.7

HIGH MUTATION RATES AND POPULATION EQUILIBRIA IN RNA VIRUS EVOLUTION. J.J. Holland, D. Steinhauer. Department of Biology, University of California at San Diego, La Jolla, CA U.S.A.

RNA viruses have long been known to have high mutation rates and we have been using methods for direct sequence determination of base substitution error frequencies. Analysis at a number of single predetermined sites of genomic RNA of in vivo replicated vesicular stomatitis virus has consistently detected polymerase error frequencies between 10^{-3} and 10^{-4}. Modificationsof these techniques have allowed for very preliminary determination of cellular RNA polymerase error frequencies at sites which were previously interactable to analysis. In addition, we have begun analyzing growth rates and competitive abilities of clonal populations of "wild type" and mutant vesicular stomatitis virus (Indiana serotype). All of our data to date are consistent with Eigen's quasispecies distribution concepts for RNA genomes, and for the evolution of viral RNA genomes, or for their relative stability under certain conditions.

S32.8

GENETIC DETERMINANTS OF ROSS RIVER VIRUS VIRULENCE FOR MICE. A. Meek, S. Faragher and L. Dalgarno, Australian National University, Canberra, Australia.

To examine the genetic determinants of Ross River Virus virulence for mice, serial passage in infant mice was used to generate variants of RRV with enhanced virulence. A plaque-purified isolate from an avirulent strain was used to begin passaging. Virus was passaged ten times in infant mice; eight independent passage series were performed. Assay of virulence by titration in mice showed that passaging resulted in substantially increased virulence compared with unpassaged virus.

The extent of genetic change between unpassaged and passaged virus was estimated as less than 0.2% by comparison of Hae III restriction digest profiles of cDNA made to virion RNA. Nucleotide sequencing was performed to localise the genetic changes. Seven of the eight passaged variants had nucleotide changes in the E2 gene which resulted in amino acid substitutions. This gene encodes the E2 protein, one of the two envelope glycoproteins of RRV. E2 is the neutralising antigen and is thought to be involved in early events in the infection of target cells. No other nucleotide changes were identified. The amino acid substitutions in the E2 protein of these variants altered the neutralisation endpoint to a number of anti-E2 monoclonal antibodies. A hypothesis is proposed relating enhanced virulence to changes in surface regions of the envelope proteins involved in virus-cell interaction.

S33.1

CLASSIFICATION AND PHYLOGENY: AN EMPIRICAL ISSUE.
Joseph Felsenstein. Department of Genetics, University of Washington, Seattle, Washington 98195, USA.

Classification has traditionally been regarded as the central task in systematics, and discussions of phylogeny are routinely considered to be discussions of classification. Even the phenetic school, which separates the two issues, considers classification to be the more important question. In the last two decades methods for inferring phylogenies have become well developed and their logic is fiercely debated, but there is often confusion as to whether it is evolutionary history or classification that is being discussed. Phylogenetic methods address issues of truth or falsity; classification is a matter of convenience. We can only agree on which approach to use if we can agree on the optimality criteria that a classification system ought to satisfy. If viral history involves both polyphyletic origins and evolution of groups of related viruses, then the issue of how we determine when a classification system is useful must be faced squarely, as a purely monophyletic system is impossible at higher levels. Under a variety of criteria involving predictability of characters, the whether the groups in the classification must always be monophyletic will turn out to depend on whether there are real and large differences in evolutionary rates between lineages, differences which affect the entire genome, rather than just small parts of it. For a variety of reasons taxonomists have not concentrated on this important empirical question, preferring too often to study philosophy rather than biology.

S33.2

BIOLOGICAL CONCEPTS IN VIRUS CLASSIFICATION. David W. Kingsbury. Department of Virology and Molecular Biology, St. Jude Children's Research Hospital, Memphis, Tennessee, USA.

For many years, it was the official position of the International Committee on Taxonomy of Viruses that "virus taxonomy...has no evolutionary or phylogenetic significance". This view is no longer tenable, and it has been officially rescinded, in the light of new information that reveals common ancestries of viruses in diverse families. Cogent examples are the unexpected conservations of virion structure, genome organization, and amino acid sequences in icosahedral RNA viruses that infect vertebrates and plants. These and other examples will be adduced to support the thesis that fundamental biological themes, such as the concept of the gene, genetic variation, and evolution, are as relevant to viruses and their classification as they are to "higher" organisms.

S33.3

THE SPECIES CONCEPT SHOULD NOT BE UNIVERSALLY APPLIED TO VIRUS TAXONOMY - BUT WHAT TO DO INSTEAD?
R. G. Milne. Istituto di Fitovirologia Applicata del CNR, Via Vigliani 104, 10135 Torino, Italy.

It is argued that the species is a natural grouping that is the reflection of a genetic structure in the population arising from a particular kind of breeding behavior. There is evidence that some viruses possess this breeding behavior and that others do not, so the species concept is applicable to some but not all viruses, and is therefore not suitable for use in a universal virus taxonomy.

The species concept and the associated idea of genera and families are rigid in the sense that they imply a given phylogenetic history. We know little about the evolutionary history of viruses, and what is coming to light through molecular studies may completely upset presently conceived ideas. This is the second reason why current application of the species concept on a wide scale would be unwise.

While the 'species' and 'non-species' viewpoints seem far apart, compromises may be possible, and some of these are discussed.

S33.4

EVOLUTION OF PLUS-STRAND VIRUSES. Rob Goldbach, Rik Eggen[1], Ab van Kammen[1], Pieter Vos[1] and Joan Wellink[1].
Departments of Virology and [1]Molecular Biology, Agricultural University, Wageningen, The Netherlands.

Viruses with single-stranded-RNA genomes and parasitic on eukaryotic cells form a very diverse group with a wide variation in genome structure and expression. This is particularly true for plant RNA viruses, which may have either segmented or nonsegmented genomes that are expressed using a variety of translation strategies. Based on these differences, and on other characteristics like host range, vector specificity, and particle morphology, the plant RNA viruses have been classified into a rather large number of groups.

During the last few years the complete nucleotide sequences of the genome of a number of plant RNA viruses, a.o. AlMV, BMV, BNYVV, CarMV, CMV, CPMV, TEV, TMV and TVMV, have been elucidated. This has created the possibility of determining possible relationships between these and other eukaryotic RNA viruses in terms of RNA and protein sequence. Such studies have revealed striking sequence homologies among nonstructural proteins of plant and animal RNA viruses, which must reflect (until recently unsuspected) evolutionary relationships. The main conclusions from these protein-sequence comparisons, which throw a totally new light on the evolution and systematics of RNA viruses will be summarized. In addition, the possible evolutionary mechanisms that may underly the interviral protein homologies detected so far will be discussed.

S33.5

EVOLUTIONARY SIGNIFICANCE OF THE TAXONOMIC DATA REGARDING BUNYAVIRUSES OF THE FAMILY BUNYAVIRIDAE. C. H. Calisher, Division of Vector-Borne Viral Diseases, Centers for Disease Control, Ft. Collins, Colorado, U. S. A.

Casals suggested that viruses belonging to a number of serogroups be placed together in a Supergroup, because of antigenic relationships between one or more member viruses of each serogroup. This Supergroup later came to be known as the genus Bunyavirus, within the family Bunyaviridae, when it was shown that viruses of the genus are biochemically, morphogenetically, and structurally similar to each other and that they have the same mode of replication. Because morphogenetic characteristics and gene coding assignments are conserved among viruses of the genus, antigenic variation (i.e. phenotypic expression) can be considered as indicative of slight changes in gene sequence. Such changes can occur as random mutations or because of selective pressure brought to bear by immune responses of the vertebrate host. Observed antigenic relationships make it reasonable to suppose that all viruses within a serogroup share common ancestry. Evidence for evolution may be seen in the close antigenic relationships among bunyaviruses isolated in sympatric situations, as well as the distant relationships among viruses found in geographic and biologic isolation from each other. Major antigenic differences may be due to reassortment among the three gene segments in cells infected with one or more closely related bunyaviruses.

S33.6

THE EVOLUTION OF HERPES AND POX VIRUSES. Glenn A. Gentry. Department of Microbiology, University of Mississippi Medical Center, Jackson Mississippi 39216-4505, U.S.A.

Recent sequence data has allowed the inclusion of several herpes and three pox viruses in four phylogenetic trees, and ancient origins for both groups may be inferred from them.

The pox tree, based on deoxythymidine kinase (dTK), includes vaccinia, variola, and monkeypox viruses, as well as chicken, Chinese hamster, mouse, and man. This tree is consistent with the origin of vaccinia virus from a recombinational event involving variola virus.

The first herpes tree includes marmoset herpes, herpes simplex (HSV), varicella-zoster (VZV), and Epstein-Barr (EBV) viruses, but it cannot be joined to the pox tree because the herpes and pox dTKs are not analogous. The other two herpes trees are based on the large and small subunits of ribonucleotide reductase (RRL and RRS). Both include HSV-1 and 2, VZV, EBV, mouse, and E. coli. The RRS tree also includes the clam (Spisula) and bacteriophage T4. These trees suggest a divergence time (7 to 8 million years ago) for HSV-1 and 2 that is near the separation of humans and apes. Finally, the herpes RRS and RRLs, unlike the pox dTKs, evolved much more rapidly than their eukaryotic counterparts, which suggests a generation time effect also noticeable in bacteriophage T4. Supported in part by NIH grant 2 S07 RR05386.

S33.7

RELATIONSHIPS OF PLANT AND ANIMAL PLUS-STRANDED RNA VIRUSES. Richard Allison and Paul Ahlquist. Biophysics Laboratory, 1525 Linden Drive, Madison, Wisconsin 53706, USA.

Many plant and animal viruses have mRNA-sense single-stranded RNA genomes. Previous taxonomy of these viruses has been based primarily on particle morphology. With the advent of sequence analysis, comparisons at the nucleotide and amino acid level have revealed previously unsuspected similarities among mRNA viruses differing in morphology and genetic organization. These similarities extend across plant and animal virus groups, and may reflect evolutionary relationships.

Among mRNA-sense viruses sequenced to date, two categories can be defined based on distinct protein homologies and RNA structures. One category includes plant como- and potyviruses and animal picornaviruses, which have a small protein linked to the 5' terminal ribonucleotide of their RNA. Another includes the plant tobamo-, tobra-, almo-, cucumo-, bromo- and other viruses as well as the animal alphaviruses, whose RNAs all have m7Gppp caps. These capped RNA viruses all share several regions of extensive amino acid homology within their nonstructural proteins. Recent and previously determined virus sequences will be discussed with regard to both long-term evolution within these major virus categories and specific genetic events, such as rearrangements, in the recent divergence of two closely related bromoviruses.

POSTER
ROUND TABLES

AFFICHES
TABLES RONDES

R1.1

SYNTHESIS IN ESCHERICHIA COLI AND IMMUNOLOGICAL CHARACTERIZATION OF A POLYPEPTIDE CONTAINING THE CLEAVAGE SITES ASSOCIATED WITH TRYPSIN ENHANCEMENT OF ROTAVIRUS SA11 INFECTIVITY. C.F. Arias, M. Lizano, S. López. Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, Mexico City 04510, Mexico.

About 45% of the rotavirus SA11 VP3 gene was inserted into a thermoinducible expression plasmid under the control of phage lambda P_L promoter. The primary translation product predicted on the basis of the plasmid construction, was a hybrid protein in which the 98 amino-terminal amino acids of phage MS2 polymerase were followed by amino acids 42 to 387 of the VP3 protein, which included, the region containing the cleavage sites associated with trypsin enhancement of infectivity. On induction, a polypeptide that had the expected mol. wt. and contained VP3-related amino acid sequences as judged by immunological criteria, was synthesized to a level representing about 15% of the total bacteria protein. When a bacterial lysate enriched for the fusion polypeptide was injected into mice, it induced antibodies which inhibited haemagglutination and neutralized SA11 rotavirus infectivity. Data will be presented about the capacity of the hybrid protein to prime the immune response in mice.

R1.2

A FIELD TRIAL FOR THE EVALUATION OF THE EFFICIENCY OF JE 5-3 STRAIN LIVE VACCINE Jian Ao et al. National Institute for the control of Pharmaceutical and Biological Products, Temple of Heaven, Beijing China.

JE live vaccine'producing with hamster kidney cell culture' has been used for prevention of JE in a epidemic region of south China. The virus titer of vaccine was log 6.0-6.5 TCID50. The vaccinated children was divided into 2 groups. Group A'aged 1-5 years' and group B'aged 6-13 years' were injected at 1 dose of 0.5ml of JE live vaccine but the group B has been vaccinated with JE killed vaccine in the past years. The JE case was identified with both HI test and clinical signs.

A total of 433,617 children were injected with live vaccine and a total of 36,068 children uninjected as a control in 1976, there was a apparently differences in the morbidity of vaccinated and unvaccinated groups of A and B. In 1977, the vaccinated groups of A and B were boostered at 1 dose of 0.5ml with live vaccine, the morbidity of vaccinated groups A and B was sharply down. After 1 year, 2years and 3 years of booster, the morbidity of vaccinated groups A and B was remained at low level. So we considered that the vaccination with JE live vaccine may be effectiveness to prevent JE of children but not satisfactory. So that we developed a higher immunogenic JE live vaccine.....14-2 strain.

R1.3

CHARACTERISATION AND COMPARATIVE IMMUNOGENICITY OF HSV-1 SURFACE PREPARATIONS OBTAINED FROM INFECTED CELLS USING A ZWITTERIONIC DETERGENT. R. Jennings and T. Quasim, Department of Virology, University of Sheffield, Sheffield, England.

Herpes hominis type-1 (HSV-1) infected cells were treated with the zwitterionic detergent Empigen, and the resulting extract partially-purified over a 20% sucrose cushion. Characterisation of this preparation using polyclonal and monoclonal HSV-1 antisera indicated the presence of several HSV-1 glycoproteins. Further purification on a sucrose density gradient resulted in fractions containing HSV-1 glycoproteins B.C.D and E and a high specific HSV activity per mg. of total protein.

Immunisation of mice with these preparations elicited high serum antibody responses to HSV-1 and marked, but lower antibody levels against Herpes hominis type 2 (HSV-2) virus. Challenge infection indicated that these animals were fully protected against HSV-1 and partially protected against HSV-2. Comparison of the immunogenicity of Empigen-extracted HSV-1 antigen preparations with those obtained using non-ionic detergents such as Triton-X-100 or Nonidet P40 clearly illustrated the superiority of the former, and suggests that further investigation into the potential of Empigen-extracted HSV-1 antigen preparations as vaccines for use against HSV infection in man could be of considerable value.

R1.4

ATTENUATION OF LUMPY SKIN DISEASE VIRUS IN CELL CULTURE.

Dr. N. O. Asagba, National Veterinary Research Institute, Vom - Jos, Nigeria.

A local isolate of lumpy skin disease virus, LSDV/Vom/74 was passaged in bovine kidney (BK) cell culture 80 times and tested in cattle to assess its degree of attenuation. No generalized reaction was produced. A local reaction (17 cm) at the site of inoculation was adjudged too large for the virus to be considered attenuated.

The 84th cell culture-passaged virus, titre Log_{10} 3.2 $TCID_{50}$/ml was grown in a non-host cell system, baby rabbit kidney cells (BRK) to attenuate it further. Limiting dilutions of the virus were grown four times in BRK cells and again three times in BK cells. This is undergoing test in cattle.

In other experiments, the 80th BK passage of the virus, titre Log_{10} 5.3 $TCID_{50}$/ml, was grown in foetal bovine muscle cell (FBMC) monolayers to compare its growth with those in BK and BRK cells. The virus took much longer to produce cytopathic effect (CPE) and was more difficult to detect in FBMC monolayers than in the other cell systems.

R1.5

STUDIES IN MONKEYS OF ATTENUATED HEPATITIS A VARIANTS. J. S. Mao, Y. Cao, D. Dong, K. Zei, Y. Lian, H. Huang. Department of Virology, Zhejiang Academy of Medicine, Hangzhou, and Institute of Medical Biology, Chinese Academy of Medical Sciences, Kunmin, China.

Strain HZ of hepatitis A virus (HAV), derived from a fecal specimen of patient 080, was propagated in primary newborn monkey kidney cell cultures. Three variants of the strain, HZM20(35), HZM20(32) and HZM20A5(32), were developed by passage at different levels and at different temperature.

Virulence for monkeys was assessed by inoculating each of variant into four monkeys. Weekly bleedings through 10 weeks postinoculation were assayed for anti-HAV titers and SGPT as well as LDH5 activities. Liver biopsies were also studied.

Seroconversion were induced in all of the inoculated monkeys. The anti-HAV titer measured by ELISA at 10 weeks postinoculation are: 40-640 with HZM20(35), 40-160 with HZM20(32) and 20-40 with HZM20A5(32). These variants showed different levels of virulence/attenuation for monkeys. Variant HZM20(35) showed no evidence of attenuation, variant HZM20(32) retained slight virulence and variant HZM20A5(32) showed no evidence of virulence for monkeys.

R1.6

USE OF A RECOMBINANT VACCINIA VIRUS HARBORING THE RABIES GLYCOPROTEIN GENE FOR VACCINATION OF WILDLIFE AGAINST RABIES. P.-P. Pastoret, B. Brochier, J. Blancou, M.P. Kieny, J.P. Lecocq, B. Languet, M. Mackowiak, P. Desmettre. Service de Virologie-Immunologie et Pathologie des Maladies virales, Faculté de Médecine vétérinaire de l'Université de Liège, Bruxelles, Belgique .Ministère de l'Agriculture, C.N. E.R., Malzéville, France. Transgène S.A., Strasbourg, France. Rhône-Mérieux, Laboratoire IFFA, Lyon, France.

Attempts to control fox rabies by vaccination with attenuated virus seem promising and we have evaluated the potential of a recombinant vaccinia virus to protect foxes against the disease (Nature, 322, 24 July 1986, pages 373-375). The administration of vaccinia virus (VV) or a recombinant harboring the rabies surface antigen gene (VVT Gg RAB) is innocuous to foxes. The recombinant virus can elicit the production of titres of rabies-neutralizing antibodies equal or superior to those obtained with conventional vaccine and, after oral administration, confers complete protection to severe challenge infection with wild rabies virus. The same in true for young cubs taken from the earth. The inoculation of the recombinant virus to adult foxes during the incubation period does not produce asymptomatic carriers of the wild rabies virus. The recombinant vaccine is also safe when given orally to wild boars (Sus Scrofa) and european badgers(Meles meles) which are the natural competitors of foxes during vaccination campaign with baits.

R1.7

IMMUNOGENICITY OF gB-1 RELATED PROTEIN OF HERPES SIMPLEX VIRUS PRODUCED IN YEAST

Y. Kino, C. Nozaki, T. Eto, K. Mizuno, R. Mori*.
The Chemo-Sero-Therapeutic Res. Ins., Kumamoto, Japan. *Department of Virology, School of Medicine, Kyushu Univ., Fukuoka, Japan.

The gB-1 gene of herpes simplex virus (HSV) was expressed in yeast. The gB-1 related protein produced in yeast (ygB-1) was purified by an immunoadsorbent and its immunogenicity was examined in mouse and guinea pig.

BALB/c mice were immunized twice with 2 µg of ygB-1 adsorbed to 50 µg of alum at 2 weeks intervals. Control mice were given only alum. One week after last injection, the mice were challenged with HSV type 1 intraperitoneally or in the corneas. In the intraperitoneal challenge, no mice immunized with ygB-1 died whereas almost all control mice died. In the corneal challenge, the control mice developed keratitis and blepharitis. The immunized mice showed little lesion.

Guinea pigs were immunized with 20 µg of ygB-1 adsorbed to 100 µg of alum with the same schedule as mice. One month after last injection, the guinea pigs were challenged intravaginally with HSV type 2. In the control animals, vesicle and ulcer formation in extravaginal area was observed. The animals immunized with ygB-1 showed no lesion development.

R1.8

IMMUNOGENICITY OF BOVINE HERPES VIRUS TYPE 1 PURIFIED GLYCOPROTEIN SUBUNITS ADSORBED TO IMMUNOSTIMULATING COMPLEXES (ISCOM). M. Trudel, F. Nadon, C. Séguin, P. Payment and G. Lussier. Centre de recherche en virologie, Institut Armand-Frappier, Université du Québec, Ville de Laval, Québec, Canada.

Bovine herpes virus type 1 (BHV-1) is an important pathogen of cattle which is responsible for infectious rhinotracheitis. BHV-1 has two major immunogenic surface glycoproteins, a 90 kDa hemagglutinin and a 130 (74 + 54) kDa glycoprotein. These proteins were purified by rate zonal sucrose density gradient centrifugation after extraction with nonionic detergent Triton X-100. For the preparation of immunostimulating complexes (ISCOMs), the glycoproteins were adsorbed, during a second rate zonal centrifugation in a sucrose gradient, to Quil A glycoside, already added to the gradient. Hemagglutinating peaks were collected and the ISCOM fractions were identified by electron microscopy. Seroconvertion of rabbits inoculated with ISCOMs was followed by neutralization, hemagglutination inhibition and ELISA assays and compared to live virus and unadsorbed viral subunits. ISCOMs gave a higher level of response than unadsorbed subunits. This high level of immunogenecity suggests their potential as a subunit vaccine.

R1.9

TWO GENETICALLY ENGINEERED PSEUDORABIES VACCINES: SAFETY, EFFICACY AND IMMUNE RESPONSE STUDIES. C. Dees[1], M. Bartkoski[1], and Saul Kit[2]. [1]Biologics Corporation/TechAmerica Inc., Omaha, NE (USA) 68134 and [2]Baylor College of Medicine, Houston, TX (USA) 77030.

Omnivac-PRV, a thymidine kinase pseudorabies virus (PRV) deletion mutant (tk-), is the first licensed live virus vaccine produced by genetic engineering. Licensure and commercial introduction of this vaccine became controversial because of unbased fears that all vaccines produced using rDNA techniques are unsafe. The safety and efficacy of Omnivac-PRV was examined in laboratory and domestic animals and compared with two commercially available tk+ PRV vaccines. A second experimental vaccine PRV(gIII-/tk-) was also studied. $10^{10.5}TCID_{50}$'s of Omnivac-PRV and PRV(gIII-/tk-) given intraperitoneally did not kill outbred or nude mice. Both tk+ vaccines killed outbred and nude mice that were given less than $10^{5}TCID_{50}$'s. Outbred mice were protected from challenge with virulent PRV by vaccination with Omnivac-PRV or PRV (gIII-/tk-). Vaccinated outbred mice had serum neutralizing antibody. Serum from mice, swine or rabbits vaccinated with gIII- PRV equally neutralized wild type gIII+ or gIII- PRV in vitro. These studies show that both tk- vaccines are efficacious and many times safer than commercially available tk+ vaccines produced by conventional methods. Deletion of the gIII glycoprotein does not reduce the ability of this vaccine virus to generate a protective immune response or virus neutralizing antibody in swine or mice.

R1.10

A CONFORMATIONAL DEPENDENT IMMUNOGEN OF INFECTIOUS BURSAL DISEASE VIRUS INVOLVED IN VIRUS NEUTRALIZATION.

K.J. Fahey, CSIRO Division of Animal Health, Private Bag No. 1, P.O. Parkville, Victoria, Australia, 3052.

Infection with Infectious Bursal Disease Virus (IBDV), a Birna virus (bisegmented double stranded RNA virus), depresses the antibody responses of young chickens. Maternal antibody, passed via the yolk sac, protects the developing chicken against infection. The duration of protection correlates with the level of neutralizing antibody post hatching.

The genome of an Australian type-1 isolate of IBDV encodes 4 structural proteins designated VP-1 (mol. wt. 92,000), VP-2a (41,000) the precursor of VP-2b (37,000), VP-3 (32,000) and VP-4 (28,000). VP-2b and VP-3 are the major structural proteins.

Native VP-2a/2b proteins were separated by affinity chromatography using specific monoclonal antibodies. The native viral proteins, but not SDS denatured proteins, inhibited the majority of virus neutralizing activity in chicken anti-IBDV sera and induced virus neutralizing antibody responses in chickens and rabbits. The chicken anti-VP2a/2b antibody passively protected susceptible chickens from infection. These proteins appear to contain a conformational epitope which is destroyed by SDS and which may be of critical importance for inclusion in a genetically-engineered subunit vaccine for breeding hens.

R1.11

ASSESSMENT OF ENVIRONMENTAL CONSIDERATIONS FOR FIELD TESTING AND LICENSING OF LIVE GENETICALLY ENGINEERED VETERINARY BIOLOGICS. G. P. Shibley, D. A. Espeseth, P. L. Joseph, R. B. Miller.
United States Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Biologics, Veterinary Services, Hyattsville, Maryland, U.S.A.

In the United States, movement of live recombinant-DNA derived veterinary biologics requires the approval of the United States Department of Agriculture-Animal and Plant Health Inspection Service. The potential risks of adverse environmental effects from the use of these products must be assessed on a case-by-case basis prior to use in open field trials. Fate of the genetically engineered organism, survival, growth, and dispersal must be considered in the licensing review. Four containment levels have been identified. The most stringent physical containment with environmentally controlled facilities and practices is included in Levels 3 and 4. An Environmental Assessment and a Finding of No Significant Impact are required before initiating quarantined field testing in Level 2. Testing in containment Level 1 will be conducted essentially in accordance with accepted scientific practices. The type of physical containment, testing, and regulatory monitoring required for movement through each containment level will be described.

R1.12

DEVELOPMENT OF A RECOMBINANT VACCINIA VENEZUELAN EQUINE ENCEPHALITIS VIRUS VACCINE. K. Kinney, J. Esposito, D. Trent, J. Mathews, J. Koehrig and B. Johnson.
Div. of Vector-Borne Viral Diseases, Centers for Disease Control, Ft. Collins, Colorado U.S.A.

Recombinant vaccinia virus containing a complete cDNA copy of the translated region of the Venezuelan equine encephalitis (VEE) subgenomic 26S mRNA coding for the VEE structural proteins has been constructed by insertion of VEE virus cDNA into the thymidine kinase gene of Wyeth strain vaccinia virus. Expression of the VEE genes is under control of the vaccinia virus 7.5 kd polypeptide promoter with the translational start site of the VEE virus 26S mRNA utilized. Cells infected with the recombinant were positive by fluorescent antibody analysis using VEE specific polyclonal and monoclonal antisera. Mice immunized with the vaccinia recombinant produced VEE specific antibodies as detected by ELISA, hemagglutination-inhibition, and neutralization of virus. Mice vaccinated with 10^{8} plaque forming units of live recombinant virus were fully protected (100% survival) or partially protected (33% survival) against encephalitis following challenge with virulent VEE virus administered intraperitoneally or intranasally, respectively. T-cell lymphocytes isolated from C3H mice immunized with recombinant virus demonstrated VEE and vaccinia specific proliferative responses.

R1.13

COMPARATIVE SEQUENCING STUDIES OF WILD-TYPE AND COLD-MUTANT INFLUENZA (H2N2)A/ANN ARBOR/6/60 AND A/LENINGRAD/147/57 VIRUSES

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[3]Moscow Research Institute for Viral Preparations, Moscow, USSR;
[4]Division of Virology and Molecular Biology, St. Jude Children's Research Hospital, Memphis, TN, USA.

Comparative sequence data has been obtained for six non-glycoprotein genes of the A/Ann Arbor/6/60 wild-type (wt) and cold-adapted (ca) viruses and for four non-glycoprotein genes of the A/Leningrad/147/57 wt and ca viruses. Comparison of the deduced amino acid sequences of the parenial and mutant virus proteins revealed nine amino acid substitutions in the A/Ann Arbor/6/60 ca virus proteins. One amino acid substitution each was predicted for the non-structural (NS_1) protein, matrix (M_2) protein, nucleoprotein (NP) and PB_2 protein; two amino acid substitutions were predicted for the PA protein and three for the PB1 protein. For the Leningrad/147/57 viruses, five coding changes were predicted. One change each in the NS1 protein, the M1 and M2 proteins and two changes in the PA protein were detected. The biological significance of mutations is discussed in light of secondary structure predictions, other available influenza gene sequences, and recombination studies published previously.

R1.14

Comparison of the Immune Response Induced by Egg and Mammalian Cell Derived Influenza B/Eng/222/82 Virus Hemagglutinins Expressed in Vaccinia Virus. P.A. Rota, M.W. Shaw and A.P. Kendal. Influenza Branch, DVD, Centers for Disease Control, Atlanta 30333

Eight hemagglutinin (HA) genes cloned from influenza B/Eng/222/82 cultured exclusively in eggs and 16 HA genes cloned from exclusively MDCK cell-grown virus were sequenced. The genome of MDCK cell-grown virus coded for HAs that were potentially glycosylated or non-glycosylated at amino acid 196, while the HAs from the egg-derived virus were non-glycosylated at this position. Complete sequence analysis of one HA clone from each subpopulation revealed no other nucleotide differences between the HAs. Two HA clones that differed only by the presence or absence of potential glycosylation at amino acid 196 were expressed in vaccinia virus. HA molecules expressed by the recombinant vaccinia viruses reacted with both monoclonal and polyclonal anti-influenza B HA antiserum and, in SDS-PAGE analysis, co-migrated with HAs immunoprecipitated from influenza B virions. Serum from A/J mice vaccinated with the recombinant virus containing the egg-derived HA had high titers of neutralizing antibody against both subpopulations of B/Eng, while mice vaccinated with the recombinant expressing the MDCK cell-derived HA had high titers of neutralizing antibody to the homologous strain and relatively low titers to the heterologous virus. These findings suggest that vaccines prepared with egg-derived influenza B viruses induce neutralizing antibodies with broader reactivity than those prepared with MDCK cell-derived virus.

R1.15

A GENETICALLY ENGINEERED SUBUNIT VACCINE AGAINST AN AVIAN IMMUNOSUPPRESSIVE VIRAL DISEASE. A. Azad, K. Fahey*, M. Brown and P. Hudson. CSIRO, Divisions of Protein Chemistry, and *Animal Health, Parkville, Victoria, Australia.

Infectious Bursal Disease Virus (IBDV), a member of the newly recognized Birnaviridae family, causes severe immunodeficiency in young chickens and makes them susceptible to other infectious agents. The genome of IBDV consists of two segments of ds-RNA. The smaller segment codes for VP1 (90kD), while the larger segment codes for a polyprotein that is cleaved to produce an N-terminal VP2 (52kD), VP4 (28kD), and a C-terminal VP3 (32kD). VP4 is involved in the processing of the precursor polyprotein.

Deletion mapping of the large genomic segment has identified the conformational epitope within VP2 that is recognized by a virus-neutralizing and protective monoclonal antibody. VP2 has been expressed to high levels in E. coli, and the recombinant protein tested as a vaccine in chickens. Antibody to the genetically engineered VP2 neutralizes the virus in vitro, and passively protects chickens from IBDV infection. The region of the IBDV genome encoding VP2 is being expressed in a wide variety of host/vector systems (bacterial and yeast), in order to produce high levels of the viral antigen in the conformation required for inducing virus neutralizing and protective antibodies in chickens.

R1.16

ANTIGENIC STRUCTURE OF TRANSMISSIBLE GASTROENTERITIS (TGE) VIRUS. L. Enjuanes, I. Correa, G. Jiménez, M.J. Bullido, C. Suñé, C. Sánchez, Centro de Biología Molecular. C.S.I.C.-Univ. Autónoma, Canto Blanco, 28049 Madrid, Spain.

B and T cell recognition of TGE virus is being studied to define the epitopes involved in the generation of neutralizing antibodies. Four antigenic sites and 10 epitopes were defined in the E2 glycoprotein of TGE virus. From them 7 epitopes were critical in TGE virus neutralization. The selection of 11 monoclonal antibody (mAb) resistant (mar) mutants allowed to differentiate three main areas on the critical site. The variability of the critical epitopes was found to be very low by studying the frequency of mar mutants isolation and their presence on viral isolates. Two critical epitopes thought to be constant by the low frequency ($<10^{-9}$) of the corresponding mar mutants were changed in mar mutants selected with different mAbs. Peptides obtained by chemical and enzymatic degradation of E2 glycoprotein were identified by their reactivity with neutralizing mAbs. The specificity of helper T-cells that cooperated with B cells in the synthesis of neutralizing antibodies is being defined with three TGE virus specific hybridomas.

R1.17

RABIES IMMUNOSOMES (GLYCOPROTEIN COATED LIPOSOMES) INDUCE AN IN VITRO SPECIFIC RELEASE OF INTERLEUKIN-2. L. Thibodeau,* G. Mercier*, P. Perrin**, P. Sureau**, and D. Oth*. Institut Armand-Frappier*, Laval, Québec, Canada; Institut Pasteur**, Paris, France.

Production of interleukin-2 (IL-2) by lymphoid cells (particularly T-helper lymphocytes), is a key event which indicates that the processing and presentation of antigen have occured, and that the subsequent cascade of immunological events is under way.

In order to establish a correlation between IL-2 production and the observed protection in pre- and post-exposure trials, following vaccination with rabies immunosome, BALB/c mice were primed by a single intraperitoneal injection of rabies antigen presented as immunosome (IMS), purified glycoprotein (G) or inactivated virus (IRV). The splenocytes of these mice were restimulated in vitro with rabies antigens, 6 days to 7 weeks after priming, and antigen-specific IL-2 production was measured using IL-2-dependent CTLL cells. It was found: 1) that, although the peak of IL-2 was at 9 days after priming, a significant amount of specific IL-2 was still produced upon restimulation up to 7 weeks after priming; 2) that the optimal amounts of IMS used for priming was from 0.05 to 0.5 µg per mouse; 3) that rabies-IMS were as active as IRV, whereas equivalent amounts of G was inactive.

R1.18

BOVINE LEUKEMIA VIRUS: BIOCHEMICAL CHARACTERIZATION AND IMMUNOGENECITY

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The iscom is a physical structure consisting of a matrix of glycoside(QA) on which glycoproteins are attached(Morein,et.al 1984). The protein gp51 of Bovine Leukemia Virus(BLV) was included in an iscom. The BLV-iscom was characterized biochemically,physically and electronmicroscopically. SDS-PAGE,immunoblotting and immuno-EM showed that gp51 was present in iscoms.

The BLV-iscom had an S-value of 19S and EM micrographs showed the cage like structure with a diameter of about 40um as previously reported for other iscoms. The BLV was harvested from the persistantly infected foetal lamb kidney celline(Van Der Maaten & Miller,1976).About 17% of total amount of gp51 present in the cell culture fluid was recovered in the iscom. The main loss of gp51 was during sedimentation of virus .

A capture ELISA utilizing monoclonal Ab's to defined epitopes was used to control the accessability and antigenecity of gp51 epitopes. Using this device it was found:

1-That iscoms prepared from virus solubilized with non -ionic detergents Triton X-100 or MEGA did not react with neutralizing monoclonals. In contrast those prepared from virus solubilized with Tween 20,Tween 80 and octylglucoside did react.

2- Neutralizing epitopes of gp51 were well exposed in iscoms in comparison to other epitopes.

It was also shown that gp51 in iscoms was highly immunogenic.

R1.19

PROPHYLAXIS AND THERAPY OF ENCEPHALOMYOCARDITIS VIRUS INFECTION IN MICE BY MONOCLONAL ANTIBODIES. F. Vlaspolder, R. van Buuren, B.J. Benaissa-Trouw, H. Harmsen, C.A. Kraaijeveld and H.Snippe.
Laboratory of Microbiology, State University of Utrecht, Utrecht, The Netherlands.

Two monoclonal antibodies (MAs) specific for Encephalomyocarditis virus (EMCV) are described; one MA UM 21.1 belongs to the IgG_{2b} isotype and the other MA UM 21.2 to the IgG_3 isotype. Analysis employing iso-electric focusing resulted in different focusing patterns. Both MAs neutralized EMCV to titers of respectively 10^6 (UM 21.1) and 10^4 (UM 21.2). No differences in neutralization titers were detected between a highly virulent and an avirulent strain of EMCV. A competitive binding assay in cell culture indicated that MAs UM 21.1 and UM 21.2 recognize different determinants on the EMCV particles. Furthermore both MAs are able to protect mice prophylactically and therapeutically against challenge with $10LD_{50}$ of the virulent strain of EMCV. One microgram of protein A sepharose purified MA UM 21.1 afforded full protection after intravenous injection 3h before intraperitoneal challenge. Even three days after an otherwise lethal infection (mean survival time: 7 days) mice could be saved by administration of MA UM 21.1 (ascites).

R1.20

CROSS-REACTIVE PEPTIDE VACCINE AGAINST INFLUENZA VIRUS INFECTION: INDUCTION OF CTL MEDIATED PROTECTION AGAINST 1934 and 1986 H1N1 VIRUS STRAINS. K.Kuwano, M.Scott[a], J.F.Young[a] and F.A.Ennis. Department of Medicine, University of Massachusetts Medical School, Worcester, MA. USA. [a]Molecular Genetics Division, Smith, Kline, and French Laboratories, Philadelphia, PA. USA.

We previously reported that adoptive transfer of a CTL clone stimulated by a hybrid protein, which contains the HA_2 subunit of A/PR/8/34 (H1N1) hemagglutinin, limited replication of A/PR/8 virus in lungs of mice. In this study we examined whether active immunization would protect mice. Mice were immunized intraperitoneally four times with 200ug of peptide without adjuvant and challenged with A/PR/8 (H1N1) or A/PC (H3N2). Spleen cells from immunized mice, stimulated with A/PR/8, lysed A/PR/8 infected target cells (e.g. 30% at an E:T ratio of 200:1) but failed to lyse A/PC-infected target cells. Immunized mice had significantly reduced lung virus titers of A/PR/8 ($10^{5.4}$ vs $10^{3.8}$, $p<.005$), but not A/PC. Peptide immunization protected mice against A/PR/8 lethal infection, compared to control mice (88% vs 0%, $p<0.008$). Immunization also protected mice against the most recent influenza H1 subtype virus strain, A/TW/86 ($p<0.005$). Neutralizing antibody was not detected in the sera of immunized mice. Thus this peptide induced subtype specific CTL which protect mice influenza H1 subtype infections. The protective epitope on the HA_2 subunit peptide has been conserved on H1 virus strains from 1934-1986.

R1.21

IMMUNE-STIMULATING COMPLEXES ('ISCOMS') AS A NOVEL HEPATITIS B VACCINE C.R. Howard, B. Sundquist, J. Allan, S. Brown and S-H Chen, Department of Medical Microbiology, London School of Hygiene and Tropical Medicine, London, UK.

Immune-stimulating complexes were prepared by the mixing of solubilized HBsAg polypeptides expressed in yeast and the triterpenoid-like compound Quil-A. Immunization of BALB/c mice with a single dose of hepatitis B iscoms in saline results in a high titre antibody response to HBsAg equivalent to that obtained in mice immunized with the original HBsAg preparation adjuvanted by adsorption to alum. Analysis of sera from mice receiving iscoms revealed high affinity antibodies directed against the major a determinants of HBsAg, and the response was still 50% of peak values after 18 months. High secondary antibody responses were observed in immunized animals previously inoculated with a sub-immunogenic dose of HBsAg indicating that hepatitis B iscoms may represent a suitable immunogen for use in individuals in whom a course of immunization with currently licenced hepatitis B vaccines has failed to produce a significant anti-HBs response. Iscoms containing synthetic peptide analogues of HBsAg determinants were prepared and immune responses to these quantitated in mice.

R1.22

PROTECTION OF THE TARGET SPECIES OF A HERPESVIRUS (PSEUDORABIES VIRUS) BY VACCINATION WITH A SINGLE GLYCOPROTEIN. E.A. Petrovskis, J.G. Timmins, D.R. Thomsen, A.M. Miller, C.C. Marchioli, R.J. Yancey, R.C. Wardley and L.E. Post. The Upjohn Company, Kalamazoo, MI USA.

Pseudorabies virus (PRV) is a herpesvirus of swine. All of the PRV glycoproteins sequenced to date have homologues in herpes simplex virus (HSV), the prototype herpesvirus. PRV produces a glycoprctein, gp50, which has been shown to be the target of neutralizing antibodies and to have homology to glycoprotein D of HSV. Due to the extensive work done on HSV gD in animal models, gp50 was evaluated as a subunit vaccine candidate for PRV. Gp50 protected mice from PRV-induced mortality either when delivered via infection with a recombinant vaccinia virus or when administered as a subunit vaccine produced in Chinese hamster ovary (CHO) cells. The gp50 synthesized in CHO cells protected swine, the natural host, from lethal infection with PRV. Gp50 has also been produced in baculovirus infected cells, as well as in other mammalian cell lines. By deleting the transmembrane and cytoplasmic domains, a secreted form of gp50 has been synthesized in various systems.

R1.23

FIRST CLINICAL TRIAL WITH SABIN INACTIVATED POLIO VACCINE. J.R. Murph, C. Grose, G. Cuthbertson, C. Mickiewicz, S. Mento, F. Cano. Dept. of Pediatrics, University of Iowa, Iowa City, Iowa, and Lederle Laboratories, Pearl River, New York, USA.

Phase I: 39 adult males with a history of previous polio immunization, were screened for antibody to poliovirus. 49% (19/39) had no detectable neutralizing antibody to at least 1 of the 3 poliovirus types at $\geq$ 1:8 and 31% (12/39) were negative at $\geq$ 1:4. 12 subjects seropositive at $\geq$ 1:4 received either 0.25, 0.5 or 1.0 ml of a highly potent inactivated poliovaccine manufactured from Sabin-strain attenuated poliovirus with D antigen content of 40:25:70 units/ml for types 1, 2 and 3 poliovirus respectively. Phase II: 9 subjects previously identified as seronegative to at least 1 type of poliovirus despite a history of adequate polio vaccination received 0.5 ml of the Sabin-strain IPV. In the first trial this vaccine was safe and highly immunogenic. All seronegative Phase II subjects also responded to the vaccine. The immune status of these individuals with waning antibody continues to be of concern, particularly in view of the recent reports of outbreaks of poliomyelitis throughout the world.

Mean Reciprocal Antibody Titers to Sabin IPV (0.5 ml)

	Phase I			Phase II		
polio type	1	2	3	1	2	3
prevaccine	39	17	58	0	0	0
7 days post	--	--	--	102	181	297
30 days post	1161	373	5344	453	181	211
9 months post	517	573	3920	--	--	--

R1.24

CHARACTERIZATION OF VACCINIA RECOMBINANTS EXPRESSING BOVINE HERPESVIRUS-1 (BHV-1) GLYCOPROTEINS gI RESP. gIII. S. van Drunen Littel-van den Hurk[1], T. Zamb[1], G. Hughes[1] and L.A. Babiuk[1,2]. [1] Veterinary Infectious Disease Organization, University of Saskatchewan, Saskatoon, Canada. [2] Molecular Genetics Inc., Minnetonka, U.S.A.

Bovine herpesvirus-1 (BHV-1) specifies at least four major glycoproteins. The genes coding for two of these glycoproteins, gI and gIII, have been cloned into the thymidine kinase gene of the vaccinia virus genome. The recombinants constitutively transcribed the BHV-1 sequences throughout the infectious cycle. By using gI-resp. gIII-specific antibodies in an immunoprecipitation assay, we demonstrated that the BHV-1 transcripts were translationally active, producing both precursor and mature forms of gI and gIII. These were the same proteins seen during BHV-1 infection and thus, they were apparently cleaved, glycosylated and transported in a manner analogous to that seen during authentic BHV-1 infections. This was confirmed by further structural analysis of gI and gIII in which the glycosidases endoglycosidase H and N-glycanase, as well as two inhibitors of glycosylation (tunicamycin and monensin) were used. In addition, both gI and gIII were expressed on the surface of cells infected with the respective recombinants.

Vaccination with either one or a combination of the infectious recombinant vaccinia viruses induced neutralizing antibodies to gI and gIII in cattle.

R1.25

RETROSPECTIVE ANALYSIS OF CRITERIA FOR VARICELLA VACCINATION

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We have already reported that a live varicella vaccine can be safely and effectively applied to the children with leukemia and other malignancies. VZV vaccine has been given to the children according to our criteria. (H.Kamiya et al, Biken J. 27,99,1984) In this paper we analysed the criteria for varicella vaccination retrospectively.

Sixty patients were vaccinated. Among them, twenty-seven patients were evaluated the immunological status at vaccination and observed completely at Mie University. All patients showed positive of two skin test at vaccination. The seroconversion rate was 100%. Five patients developed skin rash and fever after vaccination, but the symptom was mild. In 4 patients seroregression was occurred within 0.5 to 3.5 years after vaccination, and 2 cases were reinfected to natural varicella after disappearance of antibody and clinical symptoms were not severe. Eighteen patients had no adverse reaction after vaccination and VZV antibodies were maintained, no patient had episode of reinfection. There were no difference in number of lyephocyte count and the level of immunoglobulin at vaccination in each patient. We could not determine the factors related to the clinical reaction and immunoresponse, another factor may be the difference of chemotherapy at vaccination and during observation period.

In conclusion, 1) If patients are vaccinated according to our criteria, seroconversion rate and protective efficacy are excellent. 2) Among 27 patients, 5 patients had clinical reaction at vaccination and 4 patients lost VZV antibody after vaccination. 3) The number of lymphocyte and level of immunoglobulin were not related to the adverse reaction and serological titer.

R1.26

THE IMMUNE RESPONSE AGAINST A NONSTRUCTURAL IMMEDIATE-EARLY PROTEIN PROTECTS AGAINST LETHAL CMV DISEASE. U.H. Koszinowski, H. Volkmer, R. Wittek*, S. Jonjic. Federal Research Centre for Virus Diseases of Animals, 7400 Tübingen, FRG; *Institut de Biologie Animale, 1015 Lausanne, Switzerland.

Antigenspecific cytolytic T cells (CTL) protect after transfer immunodeficient mice against lethal disease caused by murine cytomegalovirus (MCMV). The dominant fraction of CTL recognize an antigen of the immediate-early (IE) phase of virus replication; CTL with IE specificity are protective. The gene coding for the antigen recognized by CTL was defined by transfection of isolated viral genes into L cells and probing of transfectants with IE-specific T cell clones. The relevant gene is the major IE gene (ie1) of MCMV and codes for an non-structural regulatory protein (pp89) which activates early gene transcription. The open reading frame of pp89 was, after excision of introns, inserted into vaccinia. Properties of the vaccinia-ie1-recombinant will be reported. The potential of virus-encoded non-virion protein to act as antigens in recombinant vaccines will be discussed.

R1.27

ANALYSIS OF ANTIBODIES TO HIV USING SYNTHETIC PEPTIDES.

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Proteins encoded by a region of the env open reading frame (PvuII to BglII) of the Human Immunodeficiency Virus (HIV) strain HTLV-IIIB have been shown to elicit neutralizing antibodies in goats. We have analyzed the reactivity of HIV positive human sera to this region using synthetic overlapping peptides. A rapid peptide synthesis method was used to make 57 peptides spanning the entire gp120 envelope amino acid sequences of HIV-IIIB, 17 of these peptides span the above region. In addition, 13 peptides were synthesized representing the different sequences of HIV-RF for the same region. These peptides were tested by ELISA using sera from HIV positive and normal individuals. Several peptides were reactive only with HIV positive sera. Selected sera were fractionated on affinity columns made of the reactive peptides and the activity of the eluted antibodies assessed. Concomitantly, peptide conjugates were injected into guinea pigs and the anti-peptide antibodies tested for the ability to bind to viral proteins and inhibit infectivity.

R1.28

REPLICATION OF FOREIGN DNA DELIVERED BY A MAREK'S DISEASE VIRUS VECTOR. J.K. Carter, R.F. Silva, R.L. Witter. United States Department of Agriculture, Agricultural Research Service, Regional Poultry Research Laboratory, E. Lansing, MI, USA.

Passage of a serotype 2 Marek's Disease Virus (MDV) at a high multiplicity of infection on chick embryo fibroblast cells resulted in the appearance of a defective virus. Digestion of the defective virus DNA with Eco RI resulted in a 4 kb amplified fragment of MDV DNA. The 4 kb fragment was cloned into pUC18 (the resulting plasmid is termed pA5) and a partial restriction map determined. When CEF were co-transfected with pA5 and DNA from the parental strain without the 4 kb fragment, both the viral and plasmid DNA in pA5 were replicated and could be stably passed to fresh CEF.

One-day old chicks were inoculated intraperitoneally with 10^4 pfu of helper virus plus pA5, helper virus only, or uninoculated. After 12 days, the chicks were bled and virus isolated from buffy coat preparations. DNA from the isolated viruses was examined by DNA hybridization for the presence of pUC sequences. Seven of 11 isolates from birds receiving helper virus plus pA5 contained pUC sequences while 0 of 15 DNA samples from chicks treated with helper virus only (13) or uninfected (2) contained pUC sequences. It can be concluded that foreign DNA carried in an MDV vector is replicated both in vitro and in vivo.

R1.29

Vaccinia virus-influenza virus hemagglutinin chimeras:expression of hemagglutinin function in cell cultures. P.F.Yang and E.L.Hedzon, Dept. of Microbiology and Immunology, University of Western Ontario,London,Ontario,Canada,N6A 5C1.

The expression of influenza virus A/JAP/57 (H2N2) hemagglutinin(H) on the surface of cells infected with a chimera of vaccinia virus(v.v.) -influenza virus H gene inserted in the v.v. thymidine kinase gene was detected by hemadsorption(HAd). Three categories of cells resulted; 1. Virus growth neg., HAd neg.; 2.Virus growth pos., HAd neg.;3.Virus growth pos.,HAd pos. Category 2 cells became HAd pos. with trypsin, chymotrypsin, and neuraminidase but not elastase treatment. Similar cells infected with influenza A/Jap/57 virus produced 2 categories; 1. Virus growth neg., HAd neg.;2.Virus growth pos.,HAd pos. Category 1 cells cannot be converted to HAd pos. by trypsin. In the latter infection, virus coded neuraminidase is present on cells, while in the case of the chimera no neuraminidase is present. The role of glycosylation and endoprotease cleavage is being tested with glycosylation and protease inhibitors. Results with a A/PR/8/34 chimera and the implications of the pathway of chimera HA maturation will be discussed with regard to the use of these viruses to produce and detect immune responses.,

R1.30

PURIFIED DUCK EGG RABIES VACCINE: CHARACTERISTICS AND CLINICAL EVALUATION.

R. Glück, A. Wegmann, J.M. Matthieu. Swiss Serum and Vaccine Institute Berne and Neurochemistry, Dept. Pediatrics, CHUV, Lausanne, Switzerland

An effective rabies vaccine must fulfill the following criteria: 1. High purity, 2. High immunogenicity and 3. Low manufacturing and purchasing costs. Such a new rabies vaccine has been developed containing the classic Pitman Moore strain grown in embryonated duck eggs. This vaccine has been highly purified and enriched in immunologically effective rabies virus glycoprotein antigen by modern chemical and physical manufacturing methods.Avian protein contamination in this new vaccine is extremely low. No detectable traces of myelin basic protein could be found by radioimmunological and immunoblotting techniques.

To evaluate the immunogenic potential of the new vaccine, vaccinations were performed in more than thousand human volunteers according to post-exposure and pre-exposure schedules, recommended by the WHO for use of potent vaccines. Immunogenicity was excellent. In addition, 61 proven rabies exposed patients survived and could be protected with the new vaccine. Tolerance was comparable with the standard human diploid cell rabies vaccine.

R1.31

AN ORAL VACCINE TO PROTECT VILLAGE CHICKENS IN ASIA AGAINST NEWCASTLE DISEASE. P. Spradbrow and A. Latif Ibrahim, Department of Veterinary Pathology and Public Health, University of Queensland, Australia and Faculty of Veterinary Medicine, Universiti Pertanian Malaysia, Malaysia.

Scavenging village chickens are an important resource in rural areas of Asia, but they are not very productive. Newcastle disease is an important cause of loss and the vaccination techniques that are effective for commercial poultry are not appropriate for use in villages.

A vaccine based on the avirulent Australian V4 strain of Newcastle disease virus has been developed. The virus has been selected for heat resistance and is fed to chickens on pellets. Adequate levels of antibody are induced and the vaccine virus spreads readily between chickens. The vaccine virus appears to have been maintained for more than a year in a small closed free-range flock. The vaccine has conferred substantial resistance against experimental contact challenge with virulent Newcastle disease virus in laboratory chickens, in village chickens kept near the laboratory under simulated village chickens and in scavenging chickens in villages. Pilot trials in Malaysia indicate good protection against natural challenge.

This study was supported by the Australian Centre for International Agricultural Research.

R1.32

FUNCTIONAL HOMOLOGY BETWEEN EPSTEIN BARR VIRUS AND HERPES SIMPLEX VIRUS GLYCOPROTEIN B AND ITS RELEVANCE FOR AN EBV VACCINE. W.L. Chan. Department of Microbiology, UMDS, Guy's Campus, London, UK

A monoclonal antibody TI57 directed against glycoprotein B (gB) of herpes simplex virus type 1 (HSV-1) shows positive indirect immunofluorescent staining with B95-8, a virus producing marmoset line immortalised with Epstein Barr virus (EBV) from the blood of a mononucleosis patient. Western blot and SDS-PAGE show that B95-8 cells contain a 115K protein which co-migrates with HSV-1 and HSV-2 gB. The gB homologue of EBV (gB-EBV), immunopurified to homogeneity using TI57 affinity column (Chan et al., J. Exp. Med. 126: 1304, 1985) cross stimulates HSV-1 immune T cells to proliferate in vitro. Mice immunised by a single subcutaneous injection of 30ug gB-EBV in saline developed significant protection against an HSV-1 challenge infection. This demonstrates that gB-EBV can be considered an EBV candidate vaccine. Molecular and immunological studies are in progress to better our understanding of the immune response to specific EBV proteins during EBV infection.

R1.33

IDENTIFICATION AND TESTING OF PROTEINS WITH POTENTIAL AS EPSTEIN-BARR VIRUS VACCINES. M. Mackett and J.R. Arrand. Paterson Institute, Christie Hospital, Withington, Manchester M20 9BX, England.

Several EBvirus open reading frames (ORF) have some homology with other herpesviruses. For example the EBvirus ORF BALF4 has homology with HSV, VZV and CMV glycoprotein gB. VZVgB and HSVgB are major components of the virion envelope and are the target for neutralizing antibodies. Also, several monoclonal antibodies directed against CMVgB neutralize the virus. We have, therefore, isolated the ORF which encodes EB virus glycoprotein gB in order to identify whether this protein should be included in EBvirus vaccines. We have inserted the EBgB gene into vaccinia virus either as the authentic protein or as an in frame fusion with the Hepatitis B virus surface antigen. These two constructs should present the EBgB to the vaccinated animals immune system in different ways. The authentic gene should be displayed on the surface of the infected cell and the fusion gene should be excreted. Vaccination of the rabbits is underway and should tell us whether the EBgB gene product is capable of raising EBvirus neutralising antibody and also give us an idea how best to present antigens to the immune system.

R1.34

BOVINE LEUKEMIA AND BOVINE LEUKEMIA VIRUS (BLV) : A MODEL SYSTEM FOR THE DEVELOPMENT OF A SPECIFIC DIAGNOSIS AND A SUBUNIT VACCINE.
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From panels of mouse monoclonal antibodies (mAbs) to BLV envelope glycoprotein gp51 and major internal protein p24, well-chosen mAbs have been selected to develop highly sensitive, specific and reproducible ELISA tests for the detection of antibodies specific of BLV infection and for the detection of BLV antigens. The "antibody" test is so sensitive that it allowed identification of one low titre antibody carrier in a pool of 80 control sera. As little as 10 pg of p24 or gp51 antigen could be clearly detected in complex media.

Study of the antigenic structure of BLV gp51 with monoclonal antibodies has allowed to define three crucial epitopes F, G, H. Combining different methods for prediction of protein antigenic determinants, results of biochemical studies and synthetic peptide approach, we wanted to precisely identify at least one crucial epitope of the gp51 antigen.

Three out of 12 peptides elicited antibodies in rabbits that specifically recognized BLV and inhibited pseudotypes to a significant level, indicating that these regions were most probably part of crucial epitopes of BLV gp51. The so defined protein regions should probably be included in the vaccine polypeptide in order to achieve maximal efficacy.

R1.35

PROTECTION AGAINST SEVERE ROTAVIRUS DIARRHEA IN VENEZUELAN INFANTS BY RHESUS ROTAVIRUS VACCINE. J. Flores[1], I. Perez-Schael[1], M. Gonzalez[1], Dorys Garcia[1], M. Perez[1], N. Daoud[1], K. Taniguchi[2], S. Urasawa[2], R.M. Chanock, and A.Z. Kapikian. National Institutes of Health, Bethesda, Maryland, U.S.A.; 1. Instituto de Biomedicina, Caracas, Venezuela; 2. Sapporo Medical College, Sapporo, Japan.

The efficacy of the rhesus rotavirus vaccine candidate (RRV) was evaluated in a longitudinal, double blind field trial in Caracas, Venezuela. 123 infants received 10^4 PFU of the vaccine, while 124 received a placebo. During a 1 year follow-up period, 21 episodes of rotavirus diarrhoea were detected, 16 in the controls and 5 in the vaccinees ($P < .05$), for a vaccine efficacy of 68%. However, in the 1-5 month old group the vaccine efficacy was 93% since only 1 episode of rotavirus diarrhea was detected in 68 vaccinees, whereas 15 such illnesses were observed in 65 controls ($P < .0001$). Moreover, for the entire study group (1-10 months of age) vaccine efficacy was 100% against the most severe rotavirus diarrhoeal episodes.

Preliminary serotype analysis of rotavirus isolates employing a monoclonal antibody assay, revealed a high prevalence of serotype 3 rotaviruses during the study period.

R1.36

PROTEOLYTIC ENZYMES IN VIVO MODIFY SIGNIFICANTLY ANTIGENIC SITES IN POLIOVIRUSES. T.Hovi, M.Roivainen and A.Huovilainen Enterovirus Laboratory, Department of Virology, National Public Health Institute, Helsinki, Finland.

Trypsin is known to cleave most type 3 poliovirus strains at the antigenic site 1 in VP1. This does not affect the infectivity of the virus but results in a considerable change in antigenic properties. Sensitivity to trypsin cleavage was a selection criterium in the antigenic drift discovered during a recent outbreak of poliomyelitis in Finland (Minor et al, submitted).

Incubation of trypsin-cleavable type 3 poliovirus strains in vitro with fluid specimens collected from human jejunum during diagnostic biopsy operations brought about a similar cleavage in VP1. We have found that sera from children vaccinated solely with the inactivated poliovirus vaccine and without contact to natural poliovirus infection neutralize poorly trypsin-treated type 3 polioviruses, in spite of moderate or high titers to intact cell culture-grown virus. Natural infection with a trypsin-cleavable strain of type 3 poliovirus, either wild or attenuated, resulted in a seroconversion to the trypsin-cleaved Sabin 3 poliovirus.

Site 1 has been identified as an antigenic site with the aid of mouse monoclonal antibodies. These results document that it is immunogenic also in man. These observations may have relevance to the optimization of antipolio immunoprophylaxis.

R1.37

SAFETY AND EFFICACY OF A NEW CHIKUNGUNYA VIRUS VACCINE: DOUBLE-BLIND, PLACEBO-CONTROLLED HUMAN TRIAL. F.J. Malinoski, G.F. Meadors, H. Ramsberg, P. Stopa, and T. Kolaczek. U.S. Army Medical Research Institute of Infectious Diseases. Ft. Detrick, Frederick, Md. 21701, U.S.A.

Chikungunya (CHIK) virus, an arthropod-borne alphavirus, causes outbreaks of a prostrating febrile illness. A live-attenuated vaccine (CHIK-181/clone 25), developed in MRC-5 human lung cells, was administered to human subjects. Prior to inoculation, these subjects had no antibody to CHIK or other alphaviruses including eastern, western, or Venezuelan equine encephalitis viruses. Clinical response, viremia, and immune response (plaque reduction neutralization and ELISA) were examined after IM immunization.

Minor clinical abnormalities were observed and included fever, arthralgias, and myalgias. However, we were unable to accurately distinguish vaccinees from controls.

No virus was isolated from plasma samples taken up to 7 days after inoculation. In vaccine recipients, PRNTs were elevated and IgM levels, measured by ELISA, rose after 10 days and continued to rise until at least day 14.

R1.38

INOCULATION OF ARGENTINE VOLUNTEERS WITH A LIVE-ATTENUATED JUNIN VIRUS VACCINE. J.I. Maiztegui, P. Feinsod*, A.M. Briggiler, C.J. Peters*, D.A. Enria, H.W. Lupton*, A.M. Ambrosio, E. Tiano, M.R. Feuillade, G. Gamboa, O. Conti, D. Vallejos, C. MacDonald*, and J.G. Barrera Oro*. Instituto Nacional de Estudios sobre Virosis Hemorragicas, 2000 Pergamino, Argentina, and *U.S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick, Frederick, Maryland 21701-5011, U.S.A.

Candid #1, a live-attenuated Junin virus vaccine candidate, was given to 14 volunteers from the endemic area of Argentine Hemorrhagic Fever. Four immune and 5 non-immune individuals received 51,000 PFU of Candid #1 and 5 non-immune individuals received placebo, intramuscularly. The non-immune volunteers were inoculated in double-blind fashion. No adverse clinical side effects attributable to the vaccine were observed in any of the volunteers during the first 7 postinoculation weeks. Routine hematological, blood chemistry, and urine tests remained within normal limits. Junin virus was isolated from blood mononuclear cells by co-cultivation from 1 non-immune vaccinee on the 9th postinoculation day. Rising titers of Junin virus antibodies were observed in 2 of 4 immune vaccinees 2 to 3 weeks after inoculation of Candid #1. Serologic conversion against Junin virus was detected 3 to 5 weeks after inoculation in 4 of 5 vaccinees. The results of this study were in complete agreement with those observed previously in American volunteers inoculated with Candid #1.

R1.39

THE PEPTIDE ROUTE TO VACCINATION AGAINST FOOT-AND-MOUTH DISEASE. M.J. Francis, D.J. Rowlands, F. Brown. Wellcome Biotechnology, Pirbright, Surrey. U.K.

Peptides representing a short segment of amino acids of the VP1 protein of FMDV induce an immune response in guinea pigs resulting in the production of high levels of virus neutralising antibodies and protection from the disease. This response is not dependent on coupling of the peptide to a carrier protein because free peptide is active when inoculated as an oil emulsion (eg, incomplete Freund's adjuvant) or encapsulated within unilamellar liposomes.

The ability of free peptide to prime the immune system for an anamnestic response to a subsequent injection of the peptide suggests that the sequence includes Th cell as well as appropriate B cell epitopes. The in vitro proliferation of T cells stimulated with nested sets of peptides supports this conclusion and suggests that the T cell epitope(s) are located in the C terminal portion of the peptide. Congenic mouse strains with different defined immune response genes showed a clear MHC restriction in their response to the peptides and experiments to modify this restriction will be described.

Nested sets of peptides and a variety of mulitmeric constructs to optimise peptide immunogenicity have been examined and the results of the experiments will be presented and discussed together with their relevance to the immunization of cattle and pigs with peptides.

R1.40

ETUDE COMPAREE DE TROIS SOUCHES DU CORONAVIRUS DE LA GASTRO-ENTERITE TRANSMISSIBLE DU PORC : CONDITIONS DE LA REPLICATION VIRALE ET DE LA SYNTHESE DES ANTIGENES STRUCTURAUX. TD.Nguyen S.Bernard, E.Bottreau, I.Lantier, JM.Aynaud.
Institut National de la Recherche Agronomique, Laboratoire de Pathologie Porcine, 37380 NOUZILLY, FRANCE
Nous avons réalisé l'étude comparative de la souche atténuée 188-SG (souche"Nouzilly") du coronavirus de la gastroentérite transmissible avec les souches Purdue-115 et D-52. Obtenue par sélection de survie en contenu gastrique de porc adulte (188 cycles d'inactivation alternés avec des passages en culture cellulaire), la souche 188-SG se distingue des 2 autres souches par les propriétés suivantes : a) un faible pouvoir infectieux; b)une multiplication tardive et modérée liée à une synthèse tardive et faible d'ARN; c) une richesse anormalement élevée en antigènes structuraux révélés par un test ELISA mettanten oeuvre des anticorps monoclonaux et polyclonaux. La tunicamycine exerce un effet un effet inhibiteur sur la synthèse des glycoprotéines E1 et E2. Parallèllement, on observe une réduction significative de la synthèse de la protéine NP non glycosylée. L'effet inhibiteur de la tunicamycine est modulé par des facteurs tenant d'une part à la cellule hote (influence du système cellulaire) et d'autre part à la souche de coronavirus. Les propriétés in vitro de la souche 188-SG sont susceptibles d'expliquer en partie ses propriétés in vivo : l'innocuité pour le porcelet nouveau-né,la résistance à l'acidité et aux enzymes digestives et l'immunogénicité pour la truie gestante.

R1.41

LACTOGENIC IMMUNITY TO TRANSMISSIBLE GASTROENTERITIS (TGE) OF SWINE INDUCED BY THE ATTENUATED NOUZILLY STRAIN OF TGE CORONAVIRUS : RELATIONSHIP BETWEEN PASSIVE PROTECTION OF PIGLETS AND TGE ANTIBODY CLASSES IN SERUM AND MILK. S.Bernard I.Lantier, E.Bottreau,J.Shirai, JM.Aynaud.
Institut National de la Recherche Agronomique, Laboratoire de Pathologie Porcine, 37380 NOUZILLY, FRANCE
An attenuated TGE virus mutant (188-SG-Nouzilly strain) making more resistant to acidity and digestive enzymes by means of a survivor selection in gastric juice of adult pig, was used for oral immunization of 27 seronegative pregnant sows. Vaccinal immunity was evaluated by resistance of newborn piglets against a severe virulent challenge and by neutralizing antibody classes separated by ion exchange chromatography and also by ELISA antibody classes. Nouzilly strain is able to induce in pregnant sows a protective lactogenic immunity for nursing piglets against TGE associated with production of high levels of TGE antibody. Highest levels of protection (70-80%) were constantly obtained in piglets when mother sows were primed and boostered by oral route. Variable results were obtained when other immunization routes (intramuscular, intramammary, conjonctival) were used (20 sows). Piglets passive protection rate is not correlated with the level of neutralizing or ELISA TGE antibody in serum and milk. Besides level of Ig classes bearing neutralizing antibodies or IgG, IgM and IgA antibody classes detected by ELISA in serum and milk were not correlated with passive protection rate.

R1.42

CROSS PROTECTION EXPERIMENTS WITH POTATO VIRUS X (PVX).
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Institute of Biology and Pedology, Far-East Science Centre of the Academy of Sciences of the USSR, Vladivostok, USSR.

As the biological method for control with virus diseases causing great damages, the weakly pathogenic virus strains are used recently rather extensively and seem to be extremely promising (Hamilton, 1985). In this respect results of our lasting studies on PVX are of interest.

We carried out a great number of labaratori and field experiments when at the beginning of vegetation the plants of some cultivars were infected by PVX weakly pathogenic strains isolated from the natural population. The complete interference to the patogenic strains of this virus was revealed in all cases and especially on early cultivars, the yield did not decrease and the efficiency of this method was observed in the subsequent tuber reproductions as well. Over a period of 10 years the increase in pathogenicity of the isolated strains did not take place.

R2.1

NUCLEAR FACTOR 1 (NF-1) BINDING SITES IN THE GENOMES OF HUMAN ONCOVIRUSES: A HYPOTHETIC ROLE FOR REINTEGRATED CELLULAR ORIGINS OF DNA REPLICATION IN MALIGNANT TRANSFORMATION

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The mechanism of cell transformation by human oncoviruses not carrying transduced onc genes has not been resolved yet. Since regulatory nucleic acid sequences might also be transduced by oncoviruses (1) we looked for the presence of binding sites for NF-1, a cellular protein which binds to adenoviral- and putative cellular origins of DNA replication (2) in the genomes of various human oncoviruses. We found that LTR sequences of HTLV-I as well as a hepatitis B virus sequence integrated into DNA of a hepatoma-derived cell line and the regulatory region of BK virus contain sequences capable to bind NF-1. We suggest that cellular origins of DNA replication acquired by oncoviruses may play a role in malignant transformation after reintegration into the cellular genome by providing new targets for cellular factors initiating DNA replication and by perturbing the temporal order of replication.

1.Minarovits J.et al.FEBS Letters 174, 208 (1984)
2.Hennighausen,L.et al. Nature 314, 289 (1985)

R2.2

TRANSCRIPTION OF 3 Kb RNA FROM BamHI-H OF MAREK'S DISEASE VIRUS (MDV) DNA IN TRANSFORMED LYMPHOCYTE CELL LINE MSB-1.

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The MSB-1 cell line carrying T-cell markers was obtained from a splenic lymphoma of chickens with Marek's disease. Since a gene for transformation might be localized in BamHI-H fragment of MDV DNA, we prepared ^{32}P-labeled MSB-1 DNA and hybridized it to BamHI clones of MDV-GA5 (received from Dr. M. Nonoyama), using the Southern blot technique. All the genome fragments were found in the viral DNA present in the transformed cells. Comparison of viral DNA from pathogenic and nonpathogenic strains of MDV revealed that amplification of BamHI-H and -D DNA fragments located in the IRL and TRL sequences occurred only in DNA of virus isolates unable to cause tumors in chickens. To further study the fate of BamHI-H in MSB-1 DNA, a cloned BamHI-H probe labeled with ^{32}P was used for hybridization with BamHI-digested MSB-1 DNA. Three distinct DNA bands of 10, 8.6 and 5.5 Kb were detected in the MDV DNA present in MSB-1 cells resembling the situation in transforming MDV isolates. Total RNA was extracted from the MSB-1 cells, electrophoresed in formaldehyde-containing gels, and hybridized to the ^{32}P-labeled BamHI-H DNA fragment. Three mRNA species (5, 3 and 2.45 Kb) were detected. Hybridization of the MSB-1 RNA with ^{32}P-BamHI-I_2 and K_1 DNA fragments located to the right and left of BamHI-H, respectively, showed that the 3 Kb mRNA is specific for the BamHI-H fragment. The 3 Kb RNA transcribed from BamHI H DNA fragment might be a transcript from a gene in BamHI-H, possibly connected with the transformed state of the cell. Supported by U.S.-Israel Agricultural Research and Development Fund (BARD)

R2.3

CHARACTERIZATION OF A CELL LINE RESISTANT TO TRANSFORMATION BY SV40.

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We have isolated a cell line, A27, which expresses a wild type SV40 large T antigen yet is not transformed. Upon infection with SV40 or transfection of SV40 DNA, these cells retain a normal growth phenotype. We have asked whether A27 cells are generally resistant to transformation by transfecting other oncogenes into the cells. The EJ-ras gene and adenovirus type 12 both transform A27. Therefore, A27 cells are specifically resistant to SV40 transformation. We interpret this as evidence that these cells are mutated in a cellular gene whose product is involved in transformation by SV40. Transfection of genomic DNA from transformation-sensitive cells does not complement the mutation, indicating that the mutation may be dominant. We have asked if we could inactivate the dominant allele by insertional mutagenesis. A27 cells were infected with Mo-MuLV^{sup}, which has the supF gene cloned into its LTR's. Transformed foci appear at a frequency of 3-5 X 10^{-7}. No foci arise in mock infected cells or in infected control cell lines. Thus, the provirus has probably inserted into the mutant allele, inactivated it, and allowed the cells to be transformed by SV40. We are currently cloning the proviral insertions, taking advantage of the supF selection, to identify the gene responsible for the transformation resistant phenotype.

R2.4

ANALYSIS OF THE TRANSCRIPTIONAL CONTROL REGION OF NON-PASSAGED BK VIRUS.

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Department of Chemical Pathology, Medical School, University of Cape Town, Cape Town, South Africa.

The nucleotide sequence of the transcriptional control region of a BK virus isolate cloned directly from human urine was compared with that of prototype BKV grown in tissue culture. Only one of the 68 bp enhancer 'repeat' elements was present but an insertion of 63 bp, not found in prototype was present. The biological activity of this region with particular reference to the 63 bp insert was studied using the chloramphenicol acetyl transferase assay. The enhancer activity of the total transcriptional control region was approximately half that of the prototype.

R2.5

TUMORIGENICITY OF pSV40 TRANSFORMED CELLS AND ONCOLYTIC ACTIVITY OF Clostridium M-55 SPORES.

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Transformation of BHK-21 cells was achieved with a PBR322-SV40 plasmid (pSV40). Newborn hamsters (less than one day old) were subcutaneously inoculated with 10^7 transformed cells. The cells induced tumors in all the inoculated animals at 18-20 days post-inoculation. Tumors were histopathologically characterized as fibrosarcoma with a growth rate of 1 cm^2/day and reached between 6-33% of the total animal weight. By Immunofluorescence they showed the viral T antigen.

When 10^6 spores of Clostridium M-55 were inoculated in hamsters already bearing tumors, either by "in situ" or endovenous route, the spores germinated, produced softening of the tumor and reduction of 50% of its growth rate, at the 3th-5th day post-inoculation. Histopatological studies showed an important cell destruction of the tumor, with presence of bacillus and only few spores. In healthy control animals the inoculated spores did not germinate and there was not evidence of any toxic effect.

When Clostridium M-55 spores were inoculated one week after the transformed cells inoculation, they caused a delay of 17 days on tumor clinical recognition.

R2.6

RESOLUTION OF A POLYOMAVIRUS-MOUSE HYBRID REPLICON VIA SITE-SPECIFIC RECOMBINATION.

A. Piché, D. Frappier, H.K. Vu, D. Bourgaux-Ramoisy, P. Bourgaux. Département de Microbiologie, Faculté de médecine, Université de Sherbrooke, Québec, Canada.

RmI is a cyclic chimera containing 1.03 copies of polyomavirus DNA and 1,628 pairs of mouse DNA joined through direct and inverted repeat sequences. It is excised from the chromosome of a transformed cell via a site-specific recombination event dependent on the activation of the viral gene coding for large T antigen. RmI is highly infectious for normal mouse cells, as it effectively yields unit-length viral DNA via intramolecular recombination. The recombination of RmI yields a second product, which is the reciprocal of unit-length polyomavirus DNA. Such resolution of RmI is dependent on the expression of a function encoded by this chimera, the viral large T antigen. At present, we are mapping on the DNA the sites required for the resolution of RmI. We are also mapping certain aberrant recombination products, which may assist us in clarifying the functions involved in recombination. The results of these experiments will be presented.

R2.7

A CELL-FREE ASSAY FOR PROSTAGLANDIN SYNTHETASE PROVIDES NEW EVIDENCE THAT THE ENZYME IS TRANSIENTLY INDUCED DURING HERPES SIMPLEX VIRUS TYPE 2 (HSV-2) CELL TRANSFORMATION. C. Krebs, M. Waite, L. Kucera, Department of Microbiology and Immunology, Bowman Gray School of Medicine, Winston-Salem, NC, USA.

Experiments were done using a cell-free assay to directly measure prostaglandin synthetase (PES) under various conditions in rat cells transformed by photo-inactivated HSV-2 or by transfection with subfragments overlapping the oncogenic Bam H1-E region of HSV-2 DNA. Nontransformed rat cells lack measurable PES regardless of treatment. Results suggest that PES is synthesized de novo following treatment of transformed cells with 0.04 uM of the tumor promoter TPA. This synthesis occurs after turnover of constitutive PES in the cells. Transformed RFS cells at low passage (<10) show a 2-fold increase in PES following TPA treatment compared to that measured in TPA-treated high passage (>20) RFS cells. Preliminary experiments using HSV-2 DNA transfected cells revealed similar results. These data correlate with our earlier 20:4 metabolism studies using intact cells. Also, the data support our hypothesis that phospholipase (20:4 deacylation) and PES activities become coupled and these activities are diminished after serial passage of HSV-2 transformed cells. In conclusion, induction of PG synthesis in transformed cells may be important in the early stages of promotion of HSV-2 induced carcinogenesis.

R2.8

SV40 DNA IN A CLONED HUMAN GLIOBLASTOMA CELL CARRIER SYSTEM. V. I. Steinberg and L. C. Norkin. Department of Microbiology, University of Massachusetts, Amherst, MA U.S.A.

The state of the SV40 DNA in a stable carrier system of A172 human glioblastoma cells was examined by Southern blot hybridization analysis. At a sensitivity of 0.1 viral genome equivalents per cell, we detected only free, apparently non-defective genomes. However, when we overexposed our autoradiograms or examined cloned cell populations, integrated viral sequences as well as aberrant forms of free viral DNA were observed. Four clones, isolated at 15 weeks, produced T antigen and displayed enhanced saturation density and plating efficiency characteristic of SV40 transformation. None of these clones produced capsid proteins or infectious virus, even upon fusion with CV-1 cells. Three of the four clones contained SV40 sequences which were present in both free and integrated states. Free aberrant DNA only, was detected in the fourth clone. These clones were susceptible to superinfection with wild-type SV40, although less so than uninfected A172 cultures. Our results suggest that integrated viral genomes might be very difficult to detect in certain uncloned cell populations.

R2.9

RESPONSE OF EPSTEIN-BARR VIRUS TRANSFORMED MARMOSET LYMPHOCYTES (B95-8 CELL LINE)TO LEAD AND OTHER AIRBORNE POLLUTANTS. E.V. Orsi and D. Koval. Biology Department, Seton Hall University, South Orange, New Jersey 07079, U.S.A..

Sub-lethal levels of lead acetate in growth medium increased the percentage of spherical forms over spindle shaped cells without decreasing the total cell number. Cytophotometric measurements of VCA immunofluorescence showed a significant increase (P= 0.05) with cells receiving 25-100 ugm/ml lead acetate than sodium acetate controls. Similar analysis for cytoskeleton components showed no difference in actin, pre-keratin, or tubulin but myosin fluorescence was more than doubled (P= 0.001) by lead. Exposure to extracts from high pollution air sample filters having 10 and 30 times the levels of lead and copper respectively than control filters reduced the fluorescence of these cytoskeleton components by more than 50 percent. Lysosome response to lead (acridine orange retention)showed a significant (P= 0.05) acceleration of dye loss with lead in contrast to increased retention with similarly treated normal and SV_{40} transformed rodent and simian cell lines. The marmoset line resembled these transformed lines in resisting growth inhibition by heavy metals not seen with untransformed controls. The marmoset line also resembled untransformed rodent and simian cells in that productive virus infection was not inhibited if exposure to air pollutants occurred after initiation of virus replication. B95-8 cells have a set of sensitive responses to the altered cell milieu not seen in our exclusively monolayer cell lines.

R2.10

IDENTIFICATION OF A TRANSCRIPT REGULATED DURING MOUSE EMBRYONIC DEVELOPMENT AND ONCOGENIC TRANSFORMATION. M. Filion, M. Lussier, A. Royal and M. Suh. Institut du Cancer de Montréal, 1560 est, Sherbrooke, Montréal, Québec, Canada H2L 4M1.

Polyadenylated RNA enriched in transformation specific sequences from hamster embryo fibroblast cells transformed by HSV-2 was used to construct a cDNA library. A cDNA clone (pKG4) contained a sequence which was upregulated in HSV-2 transformed cells and also in fibroblastic cell lines transformed by SV40 and 3-methylcholanthrene. The expression of the KG4 sequence was found to be regulated during the cell cycle. The lowest level of expression was observed in confluent cells while highest level was observed at the end of G1 and lasted only a few hours. Expression of the KG4 sequence was also examined in mouse embryos from day 8 onwards and in adult tissues. During development, KG4 is expressed at all times examined. However, there is a dramatic increase in expression on day 11. In adult tissues, a low and variable level of expression was observed. These findings suggest that the KG4 sequence is related to cellular proliferation.

R2.11

EARLY ACTIVATION OF THE PROTO-ONCOGENE c-fgr DURING EPSTEIN-BARR VIRUS (EBV) IMMORTALIZATION. M. Okano, J. Davis, and D. Purtilo. Dept. Pathology/Microbiology, University of Nebraska Medical Center, Omaha, NE 68105.

We studied c-fgr proto-oncogene expression, EB nuclear antigen (EBNA) staining and cellular DNA synthesis as events in EBV immortalization of human B lymphocytes. Peripheral blood lymphocytes (PBL) from normal adults were infected with EBV (strain B95-8). The cultured PBL were assessed for the expression of EBNA by anti-complement immunofluorescence (ACIF) at 3, 12, 24 and 48 hours post-infection (PI). EBNA was detected as early as 12 hours PI and the percentage of cells staining for EBNA gradually increased from 24 to 48 hours. EBNA was not detected in non-infected PBL controls. Cellular DNA synthesis by uptake of ^{3}H-thymidine was detected at 48 hours but not earlier. Cellular RNA was extracted to assess the expression of c-fgr of infected and non-infected PBL using slot-blot hybridization. Abundant c-fgr RNA was detected prior to expression of EBNA and cellular DNA synthesis. Expression of c-fgr was minimal in parallel studies wherein PBL were stimulated with phytohemagglutinin and pokeweed mitogen. The results of these studies strongly suggest that activation of c-fgr is an early event of EBV immortalization. Additional proto-oncogenes such as c-myc are likely activated during later stages of lymphomagenesis. Supported by PHS CA 30196, awarded by the National Cancer Institute, DHHS.

R2.12

IN VITRO IMMORTALIZATION OF COMMON MARMOSET CELLS WITH THREE SUBGROUPS OF HERPESVIRUS SAIMIRI. E. Szomolanyi[1], P. Medveczky[1,2], C. Mulder[1,2]. Departments of [1]Pharmacology and [2]Molecular Genetics & Microbiology, University of Massachusetts Medical School, Worcester, USA

The rightmost 7 kbp DNA segment of the L DNA of Herpesvirus saimiri (H.saimiri) is necessary for the oncogenicity of the virus. Based on high interstrain variability of the same DNA region, the H.saimiri strains had been classified into three groups, A, B and N (non A-non B). Representatives of all three groups were successfully used for in vitro immortalization of common marmoset (Callithrix jacchus) peripheral blood cells (PBL). H.saimiri strains of group A and N immortalized PBL from a subset of common marmosets (5 out of 13) reproducibly, however, immortalization with a strain of group B (SMHI) was achieved in only one occasion. The H.saimiri immortalized cell lines grew well in the absence of added growth factors, except cell line 473-SMHI (group B immortalized), which required IL-2 for optimal growth.

All H. saimiri immortalized cell lines contained 1-10 copies of covalently closed circular viral DNA, which could first be detected 3 weeks after infection. Cells infected but not immortalized with H.saimiri did not contain detectable amount of the viral episomes.

R2.13

EXPRESSION OF HIGH AFFINITY IL-2 RECEPTORS AND AN IL-2 LIKE GROWTH FACTOR IN CELLS TRANSFORMED BY HERPESVIRUS SAIMIRI. P. Medveczky and M. Medveczky. Dept. of Pharmacology, University of Massachusetts Medical Center, Worcester, MA 01605.

We compared growth factor production and expression of IL-2 receptors of eight cell lines transformed by viruses from three subgroups of H. saimiri that differ in DNA sequences required for oncogenicity. The experiments showed that: 1. Six of H. saimiri transformed cell lines died if cells diluted below 10^4 cells/ml suggesting that the cells secrete growth factor(s). Cells transformed by a group B virus (S295C) were semi or entirely IL-2 dependent and did not grow below $2x10^5$ cells/ml in the absence of IL-2. 2. Conditioned medium from cells transformed by group A or group non-A, non-B, viruses contained an IL-2 like growth factor that stimulated the growth of uninfected lymphocytes. In contrast, group B virus transformed cells did not secrete any factor. We detected very small amounts of IL-2 mRNA in only two cell lines. 3. All cell lines expressed mRNA and antigen of the IL-2 receptors: receptor binding assays showed high numbers of high affinity IL-2 receptors. 4. Antibodies to the IL-2 receptors inhibited the growth of all cells. 5. IL-2 stimulated group B transformed cells to a much greater extent than other cell lines. We propose that autocrine secretion of growth factor(s) and ii) expression of high affinity receptors are both involved in the process of malignant transformation by H. saimiri. The studies also suggest that induction of growth factor secretion could be the function of the hypervariable oncogenic region.

R2.15

POINT MUTATIONS IN THE SIMIAN VIRUS 40 SMALL-T ANTIGEN. P. Mulgaonkar and K. Rundell. Department of Microbiology-Immunology, Northwestern University, Chicago, IL.

The simian virus 40 (SV40) small-t antigen shares 81 amino acids at its amino-terminus with large-T and has 93 unique amino acids. The unique region of small-t is cysteine rich and deletion mutations in this region eliminate small-t associated functions. Potential roles attributed to small-t include the enhancement of SV40 mediated transformation under certain conditions of cell growth, conversion of infected CV1 cells to methylxanthine resistance and interaction with two cellular proteins. Point mutations in the unique region of small-t were provided by Dr. Joan Rutila[1] as M13 clones and are being used to reconstruct virus. Two such viruses seem comparable to wild-type SV40 in their ability to interact with two cellular proteins and to render cells resistant to methylxanthine. One of these alters val^{134} to met and cys^{153} to tyr. The other changes cys^{151} to tyr. Transformation by these viruses is being evaluated. An additional 20 mutants, each with a single amino acid change, will be presented. In addition to defining amino acid residues important to small-t function, it may be possible to define separate regions of the protein responsible for individual functions.

[1]Rutila, J., M. J. Imperiale and W. W. Brockman. 1986. J. Virol. 58: 526.

R2.17

STUDIES ON THE EXPRESSION AND FUNCTION OF THE EB VIRAL MEMBRANE PROTEIN BNLF1. Susanne Modrow, Wolfgang Jilg and Hans Wolf. Max von Pettenkofer Institute, University of Munich, Munich, FRG.

The Epstein-Barr viral protein encoded by the open reading frame BNLF1 at the right end of the viral genome was suggested as being the target for cytotoxic T-cell reaction directed against EBV-infected lymphocytes. As seen in Northern and dot blot experiments this viral gene product is transcribed in latently infected cells in addition to the nuclear antigens (EBNAs) and is the only one of these polypeptides that has characteristics of membrane proteins. Using sera against synthetic oligopeptides derived from the amino acid sequence we could show that this protein is synthesized in Burkitt's lymphoma cell lines in a truncated form lacking 138 amino acids at the NH_2-terminal end. Using antipeptide sera in immunofluorescence tests in latently infected cells, a possible reaction in about 20%-30% of the cells could be obtained; a similar amount of BNLF1-MA producing cells were identified by *in situ* hybridization using ^{3}H-cytidine labeled DNA probes. We suggest that BNLF1-MA may not be a typical latent EBV product but rather a protein expressed in 20%-30% of the cells. *in vivo* those cells are eliminated by cytotoxic T-cells; in rare cases and in combination with additional factors only the truncated form of the protein may be produced and those cells may develop into lymphomas.

R2.14

LIMITING RECOMBINATION BETWEEN THE SV40 GENOME IN COS CELLS AND INTRODUCED EARLY REGION MUTANTS. B. Phillips and K. Rundell, Department of Microbiology-Immunology, Northwestern University, Chicago, IL.

We have been attempting to produce SV40 derivatives which can encode only small-t antigen and not large-T. An SV40 deletion mutant that lacks over 1Kb of early region sequences (nt 3733-2666) was used to transfect COS7 cells. At a high frequency, wild-type virus appeared, presumably as a result of recombination of the introduced sequences with the resident genome in COS7 cells. In contrast, an SV40 derivative that contained two separate deletions (3733-2666 and 4639-4570) was grown successfully through three passages on COS7 cells. Virus productions was monitored by the appearance of the late protein VP1 in the COS cells. The resulting virus expressed small-t antigen, but not large-T antigen, following infection of CV1 cells. This suggested that the presence of two separate alterations had greatly reduced the recombination frequency. This was tested by constructing additional viruses with two deletions, or with one deletion and an inversion of portions of the early region sequences. Again, generation of wild-type virus was not observed. These results have important implications for growth of other altered viruses, such as the cDNA derivatives of polyoma virus, in cell lines that provide complementing functions.

R2.16

RECOMBINATION BETWEEN ADENOVIRUS DNA AND CELLULAR DNA IN A CELL-FREE SYSTEM:
Angelika Spies, Rolf Jessberger, Dagmar Heuss, and Walter Doerfler, Institute of Genetics, University of Cologne, Cologne, Germany.

In experiments designed to mimick elements of integration of adenovirus type 12 (Ad12) DNA into the hamster cell genome, we have developed a cell-free system. An 1.8 kbp hamster cell sequence, p7 DNA, was used as a pBR322 plasmid clone. This sequence had previously been identified as the preinsertion sequence corresponding to the site of Ad12 DNA insertion in tumor cell line CLAC1 (Stabel and Doerfler, NAR 10, 8007-8023, 1982). Nuclear extracts of BHK21 cells were prepared (ultrasonic treatment or 0.3 M NaCl extraction) and partly purified by DEAE-cellulose column chromatography, $(NH_4)_2SO_4$ precipitation and glycerol gradient sedimentation (p7 DNA-protein complex). These extracts were able to catalyze the recombination between Ad12 DNA and p7 DNA. Recombinants were isolated by transfection into $recA^-$ strains of E. coli and the Ad12 recombinants were identified by colony-hybridization with [^{32}P]-labeled Ad12 DNA. Recombinants were characterized by restriction analyses. So far, 11 recombinants from different recombination reactions were analyzed. In several of these recombinants, the cellular DNA sequence comprised the same BglI-RsaI-AvaII hamster DNA fragment. We continue to investigate whether the p7 preinsertion sequence from BHK21 hamster cells carries specific signals for the recombination with (foreign) Ad12 DNA.

R2.18

EXPRESSION AND REGULATION OF SHOPE FIBROMA GROWTH FACTOR(SFGF)
Wen Chang[1,2], Anthony Purchio[2], Shiu-Lok Hu[2]; [1]Department of Microbiology and Immunology, University of Washington, Seattle, WA98195, [2]Oncogen, 3005 1st Ave. Seattle WA98121

Shope Fibroma Virus(SFV) is a poxvirus that causes benign tumors in rabbits. It is not clear, at the molecular level, how these host proliferative response relate to viral gene expression and regulation. Previously we mapped and sequenced a EGF-like growth factor gene, namely SFGF gene, located in the unique region next to the right hand terminal inverted repeat. SFGF gene belongs to the early gene family. We determined the CAP site of the mRNA and subcloned a 350 bp DNA fragment upstream of the initiation ATG to link with chlorophenicol acetylation transferase (CAT) for the study of transcriptional controlling element of SFGF gene. Deletion mutants are currently under investigation to further localize sequences containing the promoter activity.

We are also interested in the study of SFGF. Expression of SFGF in bacolovirus is ongoing. SFV mutants deleting SFGF gene are also in progress.

R3.1

THE POSSIBLE PRESENCE OF TAHYNA (CALIFORNIA SEROGROUP) VIRUS IN THE PEOPLE'S REPUBLIC OF CHINA. Huixin Gu, H. Artsob. Department of Epidemiology, Shanghai Medical University, Shanghai, People's Republic of China (PRC); National Arbovirus Reference Service, Department of Microbiology, University of Toronto, Toronto, Canada.

Sera from 134 healthy students (20-40 year old) of City Vocational College in Xinjiang, an autonomous region in the northwest part of PRC, were studied in 1985. The sera were tested by hemagglutination inhibition (HI) against two California serogroup antigens-Snowshoe Hare (SSH) and Jamestown Canyon.

Three HI reactors were obtained. Neutralizing antibodies were confirmed in 2 of 3 HI reactors including a 24 year old male with highest titers to SSH and a 26 year old male with highest titers to Tahyna (TAH) virus.

We reported (1984) that the occurrence of at least one California Serogroup virus, closely related to SSH virus in PRC had been surmised by serological survey of residents of a suburb of Shanghai in the south of China. This study further illustrates that SSH, or a closely related virus is circulating in PRC and provides the first suggestive evidence that TAH virus may also be in PRC.

R3.2

WITHDRAWN

R3.3

WITHDRAWN

R3.4

EARLY DIAGNOSIS AND INVESTIGATION ON THE DYNAMICS OF SPECIFIC IgM AND IgG IN SERA OF HAEMORRHAGIC FEVER WITH RENAL SYNDROME (HFRS) BY INDIRECT IMMUNOFLUORESCENT ASSAY. H.L.ZHU, S.Y.XIAO, M.Y.ZHANG, T.M.ZHANG, X.M.ZHENG, C.M.HSIANG. Institute of Virology, Hubei Medical College, Wuhan, China.

This paper describes the use of indirect immunofluorescence for the assay of specific IgM and IgG in the serum of HFRS patients. KHF(76-118)virus infected Vero-E6 cells were used as antigen.

The dynamics of specific IgM and IgG in 200 serum samples of 73 cases were studied. Both of the specific IgM and IgG were soon become detectable 2-3 days after the onset of the disease, persisted for a long period.

The results showed that only 53 cases has been comfirmed. Comparring the titer of IgM and IgG of the patients there is significant difference between the mild and severe cases. In the severe cases, the titer rises rapidly and declines slowly, even more than 35 days, the titer of both types still persisted over 12800.

Using this method for the earlydiagnosis has many advantages such as easy to perform, rapid and reliable, but cannot eliminate the influence of rheumatoid factor which may exist in the sera to be detected.

R3.5

STUDY OF VIRAL ANTIGEN DISTRIBUTION, ANTIBODY RESPONSE AND CHANGE OF SOME CELLULAR IMMUNITY PARAMETERS IN RABBITS INOCULATED WITH A9 STRAIN OF EPIDEMIC HEMORRHAGIC FEVER VIRUS. Lin Yu-min, Jiang Wen-min, Liu Han-yen and Qsiang Chin-min. Virus Research Institute, Hubei Medical College, Fuchang, Hubei, PRC

Rabbits inoculated with EHFV-A9strain showed viral distribution in tissues of spleen, liver, lung, pancreas, kidney, small and large intestine in the period of 7-10 days with antigen positive peak between 9-15 days and the strongest antigen reaction in kidney at 10th day after infection by IFA.IgG antibody appeared 1st wk and the titerfrom less than 1/10 reached the peak(1/5120-1/10240) at 5th wk. T cell activities were tested by^{3}H-TdR labelling and morphology transformation, by different methods of rosette formation(including the papain method to test the auto-rosette) and by the acid-α-naphthyl acetate esterase stain method. All experiments showed a significant decrease in T cell activity rate from 1st wk after infection down to the lowest level at 3rd wk. From 4th wk it began to increase and reached the normal levelns that before infection. It is concluded that the EHFV infection in rabbits showed a reversible depression of cellular immunity without influence to theantibody production. The laboratory rabbits may be used to develope an animal model to study EHFV.

R3.6

CNS STUDY IN CEBUS APELLA INFECTED WITH JUNIN VIRUS. G. Carballal, J. R. Oubiña. Department of Microbiology. Faculty of Medicine. University of Buenos Aires. Buenos Aires. Argentina.

To determine whether the XJ-Clone 3 strain of Junín virus (JV), known to be attenuated for guinea pigs and marmosets by peripheral route, is neurovirulent for the Cebus monkey, 8 animals were inoculated by intrathalamic route with 10^5 LD_{50}

Moderate neurologic signs were observed in half of the animals and one of them died at 18 days post-infection (pi). At CNS, infectious virus, JV antigen and Ig deposition were detected at 18 and 21 days, while only antigen and Ig were found at 40 and 155 days pi. The ratio of specific antibodies in the serum vs CSF was 2, 4, 8, 64 and 80, thus suggesting a local CNS response and/or brain blood barrier alteration.

Although the Cebus is not a highly sensitive model for JV, results obtained warrant consideration when testing CNS involvement with new JV attenuated strains, proposed as candidates for human vaccine.

R3.7

MORTALITY INDUCED BY ADOPTIVE IMMUNITY IN JUNIN VIRUS INFECTED NUDE MICE. J. R. Oubiña, G. Carballal, C. Quintans, M. C. Weissenbacher. Department of Microbiology. Faculty of Medicine. University of Buenos Aires. Buenos Aires.Argentina.

Athymic mice develop a non-lethal persistent Junin virus (JV) infection with high viral titers in brain and absence of both CNS lesions and detectable antibodies. To determine the effect of cell mediated immunity in the pathogenesis of the infection, purified spleen cells from immunocompetent syngeneic animals were transfered. 61 adult Balb/c athymic mice were intracerebrally infected with 10^3 LD_{50} of JV (XJ strain). At day 15 p.i. these animals received a unique dose of normal (n) or JV sensitized (s) macrophages (Mø) or different doses of n or s T lymphocytes (L). These cells were separated by glass adherence and nylon wool column. TL were counted by indirect immunofluorescence. Transfer of n or s 10^7 Mø failed to induce mortality (0/5 each group). In contrast, mortality of infected mice reached 100% (5/5) when receiving $1.8x10^8$ sTL, 86.6% (13/15) when $0.9x10^8$ and 50% (5/10) when $0.3x10^8$ sTL were given. The mean day of death (MDD) after transfer was 7.2, 7 and 8.2, respectively. Mortality rates were identical after nTL transfer, although the MDD was delayed 2 or 3 days compared with the respective dose of sTL. All infected-non transfered, and non infected and transfered animals survived. These results show the leading role of TL in the pathogenesis of a lethal acute JV infection of athymic mice.

R3.8

INCLUSION BODIES OF HFRS VIRUS-STUDIED WITH IMMUNO-COLLOIDAL GOLD TECHNIQUE. Hung Tao,Xia Semao, Chou Zinyi,Zhao Tungxin,Hang Chansnow. Institute of Virology,Chinese Academy of Preventive Medical Sciences,Beijing,China.

In studying morphogenesis of HFRS virus,we have found three types of inclusion bodies in cyto plasm of HFRS virus infected cells(Hung T.et al. 1985,1986)This paper summarises the results obtained by using a newly developed colloidal gold EM technique. Vero-E6 cells infected with classical (A9) and atypical(R22) HFRS viruses were treated with poly- and monoclonal antibodies and then labeled with Protein A conjugated gold colloids. The following principal results were ootained:

1. As HFRS virions tnemselves,all inclusion bodies (granular,filamentous and granulofilamentous) were strongly labeled by polyclonal antibodies.
2. Monoclonal antibodies reacted differently; some did not,some did very well. To ones surprise, two neutralizing monoclonals(A35 and A25-1), that have been routinely used for immuno-fluorescent detection of HFRS virus,labeled exclusively the inclusion bodies,tne virions in close association with the inclusion bodies,were evidently free from labeling.

The study is undertaken,detail results comes.

R3.9

A STEADY-STATE PERSISTENT INFECTION IN HUMAN A-549 CELLS BY HANTAAN VIRUS. M. Matsuyama[1], T. Tamura[2] and M. Homma[3]. Public Health Research Institute of Kobe City[1], Kobe, Department of Bacteriology, Hyogo College of Medicine[2],Nishinomiya, Department of Microbiology, Kobe University[3], Kobe, Japan.

A persistently infected cell line, A-Hantaan, has been established from human A-549 cells surviving primary infection with Hantaan virus (HV) 76-118. A-Hantaan cells multiplied at a rate similar to tne non-infected A-549 cells without any cytopathic effect in the absence of an antiviral factor such as anti-HV serum or interferon for more than two years. Expression of the viral antigen typical to HV was detected in almost all cells by the indirect immunofluorescent method (IF) and was not affected by the treatment with the antiviral serum.

A-Hantaan cells produced infectious viruses which induced viral antigens by IF and form smaller plaques comparing with parent viruses on A-549 or vero E6 cells. This cell line is available as a source of viral antigens for serological studies of HV. Further characteristics of A-Hantaan cells and released viruses will be shown also.

R3.10

BIOGEOGRAPHY OF BHANJA VIRUS IN EUROPE. Z.Hubálek. Institute for Vertebrate Research, Czechoslovak Academy of Sciences, 60365 Brno, Czechoslovakia.

A comparative biogeographical analysis of 8 natural foci of Bhanja virus (Bunyaviridae) infections in Europe (Italy, Yugoslavia, Bulgaria, Czechoslovakia) showed their common, characteristic features and allowed to propose a set of "predictors" the presence of which in a landscape indicates a potential for local circulation of the virus. The predictors involve a mosaic woodland--grassland vegetation consisting largely of xerothermic communities (Quercetalia pubescentis, Festucetalia valesiacae, Brometalia erecti); regular grazing of domestic ruminants infested by ixodid ticks of the subfamily Amblyomminae (e.g. Haemaphysalis punctata, Dermacentor marginatus, Rhipicephalus bursa, Hyalomma marginatum); the submediterranean climatic pattern with a dry growing season; and the presence of at least 60% of 178 proposed bio-indicators (155 plant, 4 tick and 19 land vertebrate species). The use of suggested features has made it possible to predict the existence of foci in further European countries: Hungary, Romania, Moldavia, S.-Ukraine, Turkey, Greece, Crete, Albany, Sardinia, Corsica, Spain, Portugal, France, S.-Germany, SE.-Austria.

R3.11

MOLECULAR STRUCTURE OF TACARIBE ARENAVIRUS RNAs.

M.T. Franze-Fernandez[+], A. Ochoa[*], S. Iapalucci[+], M. Lucero[*], R. Lopez[+], O. Rey[+], N. Lopez[+], G.N. Cohen[*], M.H. Zakin[*]. [+] Centro de Virologia Animal, Buenos Aires, Argentina. [*] Biochimie Cellulaire, Institut Pasteur, Paris.

Tacaribe (Tac) virus is an arenavirus of the New World closely related antigenically to the pathogenic Junin virus. Unlike other arenaviruses which include 3 proteins - 2 glycoproteins and the nucleoprotein in the virions, Tac virions appear to contain a single glycoprotein and the nucleoprotein. One or two additional polypeptidesof 79 and 105 kd are visualized in Tac virus-infected cells. For studying the molecular biology of Tac virus, the viral RNAs were cloned. The nucleotide sequence of the S RNA revealed that in Tac virus - like in other arenaviruses - the S RNA is ambisense. The glycoprotein precursor (GPC) is encoded in the 5' region of the genome in the viral-sense sequence and the nucleoprotein in the 3' region, in the viral-complementary sequence. The intergenic region can be partially arranged in a hairpin configuration. As in other arenaviruses, Tac GPC has two hydrophobic domains. The dibasic conserved aminoacid sequence that in other arenavirus GPC_2 appears between positions 256 to 271 was not found in Tac GPC. About 90% of Tac virus L RNA has been cloned. The nucleotide sequence so far determined revealed an open reading frame in the virus-complementary strand.

R3.12

ERVE VIRUS, AN UNCLASSIFIED AGENT POSSIBLY BUNYAVIRIDAE, ISOLATED FROM SHREWS IN FRANCE. C. Chastel, A.J. Main, M.C. Quillien, P. Richard, G. Le Lay, J.C. Beaucournu. Universities of Brest and Rennes, France, and Y.A.R.U., New Haven, Connecticut, USA.

A 'new' virus, represented by three apparently identical strains, was isolated in Western France from tissues of the white toothed shrew *(Crocidura russula)*. This virus adapted only to suckling mouse brain and VERO cells, clone 6, exhibiting very low infectious titers. Both morphology and morphogenesis of the agent were compatible with Bunyaviridae, but no serological relationship was demonstrated with any known Bunyaviridae (including hantaviruses), other arboviruses or other animal viruses. CF antibody was demonstrated in France from rodents *(Apodemus sylvaticus)*, insectivores *(Talpa europaea)*, artiodactyles *(Sus scrofa, Cervus elaphus,* sheep*)* and charadriformes *(Larus argentatus)*. Serosurveys on blood donors living in Western France are in progress, in order to determine the ability of this unclassified virus to infect man in nature.

The virus was provisionally named 'Erve virus' following the geographical location of the original isolations: the Erve River Valley in Mayenne.

R3.13

TRANSLATION OF THE M RNA SEGMENT OF THE BUNYAVIRUS GERMISTON.
N.Pardigon, P.Vialat,S.Gerbaud,M.Girard and M.Boulov
Unité de Virologie Moléculaire, UA CNRS 545,Institut Pasteur 25 rue du Dr.Roux, 75105 Paris 5france)

Lysolecithin-treated cell extracts from both BHK21 and HeLa cells have been used to translate in vitro Germiston virus specific proteins. In vitro translation of m RNAs from infected cell extracts led to the synthesis of the four structural proteins, L, N G1 and G2 and the non structural p12 protein (NS_S). The 2 polypeptides G1 and G2 were synthetized in a glycosylated form. When purified viral S mRNAs were added to micrococcal nuclease treated cell extracts from infected or uninfected cells, the N and p12 proteins were translated. In contrast, no product was synthetized when viral M mRNAs were added.Because low amounts of purified M mRNA were available, we are presently synthetizing M mRNA in vitro from a plasmid containing the Germiston M cDNA inserted downstream from the promotor of the T7 phage RNA polymerase. In addition, sequence data of M cDNA of Germiston virus indicates that some regions share a degree of homology greater than 60% with the Bunyamwera virus M segment, the prototype of this serogroup .

R3.14

IN VIVO AND IN VITRO TRANSCRIPTS OF THE GERMISTON BUNYAVIRUS.
M.Boulov,P.Vialat,S.Gerbaud,N.Pardigon and M.Girard
Unité de Virologie Moléculaire, UA CNRS 545,Institut Pasteur, Paris (France)

The complete sequence of the S segment of Germiston virus is 980 nucleotide long with the first 15 bases at the 3'and 5' ends complementary to each other. Three overlapping open reading frames (ORF) were identified in the viral complementary strand.The first two ORFs code for the N nucleoprotein and the NS_S non structural polypeptide, respectively. The presence of the third potential gene product of 75 amino acids in infected cells is presently investigated using antibodies raised against a region of this polypeptide synthetized in E.coli as a β-galactosidase fusion protein .

By S1 mapping analysis we showed that the S mRNA lacks approximately 115 nucleotides of the genome at its 3' end. The 5' end of the S mRNA was analyzed by primer extension. The cDNA products which contain the copy of the 3' end of the genome extended by 10-15 bases of unknown origin were cloned into pBR327 in order to determine and compare these additional viral non coded sequences from several S mRNA molecules.

Transcription products synthetized in vitro under various conditions were analyzed using S RNA specific probes . Their 5' and 3' ends were analyzed respectively by S1 mapping and primer extension.

R3.15

CLONING AND SEQUENCING OF THE M RNA OF LA CROSSE VIRUS.
L.J. Grady, M.L. Sanders and W.P. Campbell, Wadsworth Center for Laboratories and Research, New York State Department of Health, Albany, N.Y., U.S.A.

La Crosse virus belongs to the California serogroup of the family Bunyaviridae. The proteins encoded by the M segment of the genomic RNA, two glycoproteins and a non-structural protein, have been implicated in many of the important biological characteristics of these viruses. Therefore, as a first step toward gaining further insight into structure-function relationships in this group of viruses, the M RNA segment of an isolate of La Crosse (LAC) virus has been cloned and at present, >70% of its sequence determined. Based on current data, there is about 80% sequence homology between the M RNA of LAC and that of the closely related snowshoe hare (SSH) virus, whose M gene sequence has been reported by Eshita and Bishop (Virology 137, 227-240, 1984). Likewise, it is also evident that all of the potential asparagine-linked glycosylation sites are conserved between these two viruses.

A detailed comparison of the LAC and SSH M RNAs, as well as of the polyproteins which they encode, will be presented. For the polyproteins this will include the use of methods for predicting secondary structure, hydrophilicity and amphipathicity for the purpose of calling attention to residues that may be associated with antigenicity.

R3.16

PACKAGING OF LA CROSSE VIRUS RNA IN VITRO. M. Hewlett, N. Acheson, and D. Kolakofsky. Department of Microbiology, University of Geneva Medical School, Geneva, Switzerland.

The genome of La Crosse virus consists of three negative-sense RNAs, designated L (large), M (middle), and S (small), each of which is associated with the viral N protein to form helical nucleocapsids. Sequence homologies at the 5' and 3' termini of the three genome RNAs suggest that the first 8 to 13 nucleotides may be necessary and sufficient to allow genome replication and packaging into nucleocapsids. In addition, the 5' and 3' ends within each genome segment are complementary over a length of 25-30 nucleotides and therefore can form base-paired structures, which may explain the circular nature of these nucleocapsids. We wish to understand the nature of packaging and replication signals used by this virus. We have begun by constructing a plasmid which can be transcribed in vitro to produce an RNA whose first 25 nucleotides are identical to the 5' end of the S antigenome segment. This plasmid is based on the vector pPHI, designed by P. Ahlquist (Mol.Cell.Biol. 4, 2876), which directs E. coli RNA polymerase to begin in vitro transcription at the first nucleotide of a DNA fragment inserted at a unique SmaI site. We are examining the ability of such transcripts to form stable complexes with N protein in vitro. We also plan to transfect susceptible cells with these transcripts in order to determine whether replication and packaging can be carried out in the cell.

R3.17

MOLECULAR STUDIES OF LCMV GENE EXPRESSION IN ACUTE AND PERSISTENT INFECTIONS. F.V. Fuller-Pace, S.J. Francis, M.J. Buchmeier and P.J. Southern. Department of Immunology, Research Institute of Scripps Clinic, La Jolla, CA, USA.

The LCMV genome consists of two single-stranded RNA segments: L and S. The S RNA encodes an internal nucleoprotein (NP) and two surface glycoproteins (GP-1 and GP-2). Sequencing and hybridization experiments have shown that the genomic S segment has an unusual ambisense coding arrangement where NP mRNA is complementary to the genomic S RNA and GP mRNA is in the same sense as the genomic S RNA. We have identified a 200kd protein, encoded by a genomic complementary L mRNA, that is thought to be part or all of the viral RNA dependent RNA polymerase. We have analyzed total intracellular RNA in Northern blotting experiments with strand-specific probes to monitor and discriminate between replication and transcription during acute infection. NP mRNA accumulates prior to newly synthesized genomic S RNA, GP mRNA and L RNA providing direct experimental support for a hypothetical model of arenavirus S gene expression (Auperin et al. J Virol. 52:897-904, 1984). Persistent LCMV infections in vivo are characterized by accumulation of both genomic sized RNAs and a complex population of hetergeneously-sized lower molecular weight RNAs that may be linked to the establishment and maintenance of persistent LCMV infections.

R3.18

MAPPING CTL EPITOPES OF LYMPHOCYTIC CHORIOMENINGITIS VIRUS. J.L. Whitton, P.J. Southern, H. Lewicki and M.B.A. Oldstone, Department of Immunology, Scripps Clinic and Research Foundation, La Jolla, CA, USA.

We have cloned residues 1-363 of the glycoprotein (GP) of lymphocytic choriomeningitis virus Armstrong strain (LCMV-ARM), expressed it in a vaccinia virus (vv) vector, and analyzed the ability of recombinant vv to direct MHC-restricted LCMV specific cell lysis by cytotoxic T lymphocytes (CTL). We observe that, firstly, GP is a major target antigen for MHC class I restricted CTL on the $H2^{bb}$ background. Secondly, in LCMV the GP provides a target for CTLs induced by a serotypically distinct strain of LCMV (Pasteur), unlike in several other viruses where internal (non glycosylated) proteins are the major antigens directing CTL recognition between different viral strains. Thirdly, the ability of the GP to be seen by CTLs depends on the MHC background on which it is presented; $H2^{bb}$ cells are efficiently lysed while $H2^{qq}$ and $H2^{dd}$ cells remain intact.

We have extended the epitope mapping on $H2^{bb}$ by constructing truncated GPs, and by amassing a library of 18 CTL clones. We find that there are 3 families of CTL clones; one (1/18) fails to recognize GP residues 1-363, a second (2/18) recognizes an epitope between residues 1-218, and the third, (15/18) sees GP residues 1-293 but not 1-271.

R3.19

SUSPENSION CULTURE OF HYBRIDOMA CELLS SECRETED MONOCLONAL ANTIBODIES AGAINST HFRS VIRUSES.
G.F.Zhu, M.J. Zhang, F.Q.Li, J. Wang, X.T. Yuan. Institute of Microbiology & Epidemiology, Academy of Military Medical Sciences, Beijing, China.

Brains of mice infected with hemorrhagic fever with renal syndrome viruses (HFRSV) were inactivated by γ-radiation, with which the BALB/c mice were immunized securely. By the fusion of the splenocytes of these immunized mice with myeloma cell SP2/0, we have obtainedd the hybridoma cells secrted monoclonal antibodies (McAb) against HFRSV. These hybridoma cells adapted to growth in Eagle's medium supplemented with substitutes of calf serum, could be propagated well in impeller agitated fermenter, it showed 10 folds increase in population density during 4 days incubation. The culture fluid contained McAb with titer of 1:128-1:256. By use of cytodex or DEAE-cellulose as microcarrier in suspension culture, the cell density and Ab titer increased more than 10 folds. A small amount of HFRSV ($TCID_{50}$) could be detected by ELISA sanwich method using our prepared McAb.

R3.20

PRIMARY CTL RESPONSE IN MICE IMMUNIZED WITH VACCINIA VIRAL VECTORS EXPRESSING RIFT VALLEY FEVER VIRAL GLYCOPROTEINS.
M.A. Balady. Disease Assessment Division, USAMRIID, Fort Detrick, Frederick, MD 21701-5011, USA

The cellular immune response to Rift Valley fever virus (RVFV) was tested in mice infected with either RVFV or vaccinia vectors expressing glycoproteins derived from the M RNA segment of the RVFV genome. RVFV antigens, as measured by antibody-mediated complement-dependent cytolysis and fluorescent antibody visualization, were expressed in vitro on the surface of cells infected with either RVFV or vaccinia recombinants. The Lunyo virus strain of RVFV produced greater numbers of cells expressing cell-surface antigen than the ZH-501 strain. Vero cells and P-815 cells did not produce appreciable amounts of cell surface antigen, while L-929 cells did.

Cytotoxic T cells (CTL) and neutralizing antibodies were generated after immunization with vaccinia vectors containing RVFV genomic information and in Lunyo-infected mice. Four vaccinia vector recombinants containing portions of the M segment of the RVFV genome were tested. CTL's were demonstrated in both spleen and lymph node cells. Maximal response with lymph node cells occurred 6 days after immunization and waned to 50% of the peak response by day 13. This response was attributable to Thy 1.2+, Lyt 1-,2+,3+ cells and showed MHC restriction. The highest levels of cytotoxic activity were elicited by vaccinia vectors containing the largest proportion of the M segment of RVFV.

R3.21

AN INDIRECT IMMUNOFLUORESCENT ASSAY FOR THE DETERMINATION OF THE IN VITRO ANTIVIRAL EFFECT OF RIBAVIRIN, SELENAZOFURIN, 3-DEAZAURIDINE, AND (S)-DHPA AGAINST PICHINDE VIRUS.
Noah J. Burns, B. B. Barnett, J. H. Huffman and R. W. Sidwell Department of Animal, Dairy, and Veterinary Sciences, Utah State University, Logan, UT 84322 U.S.A.

Pichinde virus (PCV), a member of the family Arenaviridae, is used in hamsters as a model for hemorrhagic disease. However, PCV produces only marginal cytopathogenic effect (CPE) in vitro, developing 10-14 days post-infection (p.i.). Antiviral assays against PCV thus often utilize plaque reduction (PR) which requires relatively large amounts of drug and is slow to reach an acceptable endpoint. An indirect immunofluorescent assay (IIFA) for PCV has been developed for antiviral drug assays against PCV. The assay is performed by adding anti-PCV serum to virus-infected cells at 24 hr p.i., followed by fluorescein-labeled goat anti-mouse antibody. Infected fluorescing cells are counted with an epifluorescence microscope. Selenazofurin (2-β-D-ribofuranosylselenazole-4-carboxamide), ribavirin (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide), 3-deazauridine, and (S)-DHPA ([S]-9-[2,3-dihydroxypyropyl]adenine) were tested against PCV using this IIFA. The 50% effective dose (ED50) for each drug tested was: Selenazofurin, 1.0 μg/ml; ribavirin, 4.5 μg/ml; 3-deazauridine 7.5 μg/ml; and (S)-DHPA, 505.5 μg/ml. The ED50 of ribavirin using inhibition of marginal CPE was 6.0 μg/ml and using PR was 2.5 μg/ml, indicating IIFA was as sensitive as these other tests.

R3.22

CHARACTERIZATION OF TACARIBE VIRUS GENOME AND VARIANTS RESISTANT TO NEUTRALIZATION BY MONOCLONAL ANTIBODIES
M. W. Salter, L. Allison and C. R. Howard, Department of Medical Microbiology, London School of Hygiene and Tropical Medicine, London, UK.

Neutralizing monoclonal antibodies to Tacaribe virus recognize two epitopes on the outer envelope glycoprotein. Antibody to site A was used to generate variants resistant to neutralization which can be differentiated into 2 or more groups by the extent of recognition by antibodies to site B. A further generation of variants at site B have been produced and similarly analyzed for reciprocal changes at site A. In order to define these changes, the S RNA strand of the parental virus has been cloned into pBR 322. Seven clones 0.5 to 1.1 Kb in length were selected, 3 of which hybridized with the Pichinde N gene clone A3-89. Sequence data of clones covering both the Tacaribe N and putative GPC genes will be presented together with preliminary characterization of variant virus RNA.

R3.23

NUCLEOTIDE SEQUENCE ANALYSES OF THE M AND THE S GENOME SEGMENT OF HANTAAN VIRUS.
D. Yoo, C.Y. Kang. Department of Microbiology and Immunology, Faculty of Health Sciences, University of Ottawa, Ottawa, Ontario, Canada K1H 8M5

The tripartite genomic RNA segments of Hantaan virus 76-118 strain, the prototype virus causing haemorrhagic fever with renal syndrome (HFRS), were cloned and sequenced. The complementary DNA clones were grouped by Southern blot hybridization. Each group of cDNA was assigned to a genomic RNA segment by Northern blot analyses. Viral specific, synthetic oligonucleotides were used to obtain the clones representing 3' and 5' terminal sequences. Physical maps of all three genome segments were constructed on the basis of the cross hybridization and Northern blot analyses. The nucleotide sequence of the M and the S segments have been determined by dideoxy chain termination method after subcloning into M13mp18 and M13mp19. The M RNA segment contains 3616 nucleotides and has a potential to encode both glycoproteins,G1 (72K) and G2 (56K) whereas the S RNA segment contains 1694 nucleotides which has one large open reading frame capable of coding for the nucleocapsid protein (45K). The coding stategies of the M and the S RNA segments will be discussed.

R3.24

CRYO-ELECTRON MICROSCOPY OF ENVELOPED VIRUSES.
B. V. V. Prasad[1], G. J. Wang[1], J. P. M. Clerx [2], Y. Talmon[3], W. Chiu[1], M. J. Hewlett[2] and K. V. Holmes[4]. Dept. of Biochemistry[1], Dept. of Molecular and Cellular Biology[2], Univ. of Arizona, Tucson, AZ 85721, Dept. of Chemical Engineering[3], Technion, Haifa, 3200 Israel, and Dept. of Pathology[4], Uniformed Services Univ. of the Health Sciences, Bethesda, MD 20814.

We have undertaken the cryo-electron microscopy of two enveloped viruses: LaCrosse virus (LAC) of Bunyaviridae family and mouse hepatitis virus (MHV) of coronaviridae family. In this method unfixed and unstained viruses are observed in an unsupported thin layer of vitreous ice. This method is particularly useful for enveloped viruses which are often extremely fragile and do not withstand the drastic changes in their native environment caused by negative staining, drying or fixation. Electron images of LAC and MHV embedded in vitreous ice clearly reveal structural features such as glycoprotein spikes and bilayer membranes. In contrast to the pleomorphic appearance in the negatively stained preparation, these viruses exhibit uniform spherical shape in ice. Images of the ice embedded specimens show a significant variation in the sizes of both LAC and MHV particles. The size (including the length of the spikes) varies from 75 to 115 nm for LAC and 100 to 135 nm for MHV. Irrespective of the size, the thickness of the bilayer is measured to be about 4 nm in both viruses and the length of the spikes is about 10 nm for LAC and 13 nm for MHV. Similar studies are in progress on Pichinde virus.

R3.25

PROTECTIVE EFFICACY OF JUNIN-REACTIVE MONOCLONAL ANTIBODIES. D.Y. Pifat, R.H. Kenyon, A. Sanchez*, and C.J. Peters. U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland 21701-5011, U.S.A., and *Center for Disease Control, Atlanta, Georgia 30333, U.S.A.

Twenty-one independent hybridomas were produced using mice immunized with a virulent strain of Junin virus, the etiologic agent of Argentine hemorrhagic fever. These monoclonal antibodies were characterized in terms of their biological properties and were used to identify specific antigenic determinants capable of eliciting protective immunity. Eight monoclonal antibodies were found to react with the 38,000 MW surface glycoprotein, but only seven of these had the capacity to neutralize virus in vitro. Of the seven neutralizing monoclonal antibodies, only five were capable of lysing Junin-infected cells in the presence of complement. All remaining monoclonal antibodies reacted with the nucleocapsid protein and were unable to neutralize virus. Although none of the monoclonal antibodies discriminated between virulent and attenuated strains of Junin virus, either by fluorescent antibody or ELISA assays, many of them had the capacity to distinguish between various members of the Arenavirus family. The ability of the monoclonal antibodies to provide protection in vivo most closely correlated with their ability to lyse infected cells in the presence of complement and not with their ability to neutralize virus in vitro.

R3.26

INDUCTION OF NEUTRALIZING ANTIBODIES TO RIFT VALLEY FEVER VIRUS WITH SYNTHETIC PEPTIDES. J. Smith, L. Hodgson, M. Dombalagian*, and A. Komoriya*. Virology Division, USAMRIID, Fort Detrick, Frederick, MD 21701-5011, U.S.A., and *Meloy Laboratories, Springfield, VA 22151, U.S.A.

Previous studies with monoclonal antibodies and antigen-specific polyclonal sera have established that 1) both G2 and G1 glycoproteins of Rift Valley fever virus display epitopes capable of inducing antibodies which neutralize infectivity in vitro, 2) only G2-specific antibodies efficiently protect experimentally challenged animals, and 3) the sequences specifying the antigenic determinants of existing protective anti-G2 monoclonal antibodies are clustered within a 135 amino acid sequence on this molecule. In an effort to define additional neutralization epitopes, and to determine whether synthetic peptides could induce neutralizing antibodies, nine peptides corresponding to G2 sequences within the 135 residue sequence were synthesized, coupled to KLH by carbodiimide condensation, and subsequently used as primary and secondary immunogens in rabbits. The sequences (9 to 14 residues) were selected on the basis of hydropathy characteristics, predictions made from the Chou and Fasman algorithm, and previous epitope mapping studies. Resulting antisera were evaluated in anti-peptide, anti-virion, and neutralization assays, and by immunoprecipitation, western blot, and immuno-fluorescence procedures. Most of the peptides induced antibody reactive with native viral antigen, and one peptide induced high-titered neutralizing antibodies.

R3.27

INITIAL CLINICAL ASSESSMENT OF HUMANS INOCULATED WITH A LIVE-ATTENUATED JUNIN VIRUS (JV) VACCINE. C. MacDonald, K. McKee, C. Peters, T. Cosgriff, F. Feinsod, and J. Barrera Oro*. U.S. Army Medical Research Institute of Infectious Diseases, and the Salk Institute, Government Services Division*. Fort Detrick, Frederick, Maryland 21701-5011, USA.

The safety and reactogenicity of Candid #1, a live-attenuated JV vaccine against Argentine hemorrhagic fever (AHF), has been evaluated in 41 adult volunteers, aged 19-62, utilizing a variety of dosage and inoculation route combinations. The initial four volunteers received 2.9 x 10^4 PFU of vaccine subcutaneously; the remainder were inoculated intramuscularly with doses of 5.0 x 10^4 PFU (N=18), 1.3 x 10^4 PFU (N=15), and 2.0 x 10^3 PFU (N=4).

Symptoms, physical signs, and clinical laboratory studies, including routine hematology and urinalysis (N=41); total T cells, T4/T8 ratios (N=37); coagulation studies, serum immunoglobulins and complement (N=13); muscle enzymes, hepatic and renal functions (N=17), were monitored for 30-180 days postinoculation. Candid #1 was well tolerated clinically; minor local or systemic signs observed could not be definitively attributed to the vaccine. Laboratory parameters did not differ significantly among experimental and/or control groups. Our studies suggest that Candid #1 induces a low and acceptable level of clinical and laboratory reactions in humans.

R3.28

REDUCED RECOVERY OF RIFT VALLEY FEVER VIRUS ASSOCIATED WITH ASSAY OF MOSQUITO LARVAL POOLS. M. J. Turell, R. F. Tammariello, Jonathan F. Smith, and C. L. Bailey. USAMRIID, Fort Detrick, Frederick, MD, USA.

Inclusion of as few as 4 uninfected mosquito larvae significantly reduced the detection of Rift Valley fever (RVF) virus by plaque assay for homogenates of mosquito larvae. The presence of uninfected larvae reduced RVF virus plaque formation on all cell strains tested (Vero, BHK-21, and LLC-MK_2), and also reduced chikungunya and La Crosse virus plaque formation on Vero cells. Heat inactivation (56°C for 45 minutes) of larval suspensions greatly reduced this inhibitory effect. Reduction of viral titer depended on the number of larvae per pool and the temperature and time of incubation prior to assay. Centrifugation of a larval suspension in a sucrose gradient (15-55% w/w) for 14 hours at 130,000 x g resolved 3 separate peaks of plaque-inhibiting activity.

Our failure to recover virus from numerous larval pools (some of which contained as few as 5 larvae) known to contain RVF virus, indicates that plaque assays may not be appropriate to examine larval pools for transovarial transmission of RVF and other viruses.

R3.29

THE ENTRY OF BUNYAMWERA VIRUS INTO CELLS AND ITS NEUTRALIZATION BY SPECIFIC ANTIBODY. W. James and I. Price. Sir William Dunn School of Pathology, University of Oxford, OX1 3RE, ENGLAND.

Bunyamwera virus (Bunyaviridae) is believed to infect cells after receptor-mediated endocytosis by pH-dependent fusion of the viral envelope with an endosomal membrane. In principal, neutralization by specific antibody may involve inhibition of any of the steps in this process. We used three neutralizing monoclonal antibodies, adaptation to alternative host cells and to high or low endosomal pH values to dissect the infection process.

By using genomic recombinants between Bunyamwera, Batai and Maguari viruses, we showed that, as expected, ability of virus to bind neutralizing antibody depended upon possession of homologous M (glycoprotein-encoding) RNA segment. However, for one neutralizing MAb, neutralization *per se* depended upon possession by the virus of both M and S (nucleoprotein-encoding) RNA segments. This suggests that an essential step of cellular infection depends upon glycoprotein-nucleoprotein interactions.

A second approach confirmed that possession of an immunologically unaltered neutralization epitope did not always confer sensitivity to the corresponding antibody. Only about 50% of strains resistant to a given neutralizing MAb had a measurably decreased affinity for that antibody.

Functionally-labile neutralization epitopes may protect key domains from antibody selection.

R3.30

COMPARISON OF THE NUCLEOTIDE SEQUENCE AND GENE ORDERS OF HZ2 AND HANTAAN VIRUS GENOME M-RNAs.....P.W. Coyle, C. Hang, M.P. Kiley, A. Sanchez, D.A. Auperin, and J.M. McCormick.....Centers for Disease Control, Special Pathogens Branch, Atlanta, GA 30333, U.S.A.....

Haemorrhagic fever with renal syndrome (HFRS) is caused by members of the recently classified Bunyavirus genus, the Hantaviruses. The type species of this genus, Hantaan virus 76-118, is transmitted by the rodent Apodemus Agrarius, and is associated with a severe form of HFRS. The M-RNA of this strain has recently been shown, as with other bunyaviruses, to code for 2 virus specific envelope glycoproteins which are likely to play a central role in the pathogenesis of HFRS disease. We are in the process of sequencing the M-RNA of a Rattus derived strain, HZ2 from the People's Republic of China, which causes a milder form of the disease. To date we have sequenced the first 1081 bases from the 3' end, and comparison of the nucleic acid and amino acid sequences to those of Hantaan virus shows a 54% and 66% homology, respectively.

R3.31

RISK FACTORS FOR CONGO/CRIMEAN HEMORRHAGIC FEVER VIRUS HUMAN INFECTIONS IN SOUTH AFRICA.....S.P. Fisher-Hoch H. Kustner, K. Swanepoel, A. von Middlekoop, J.B. McCormick.....Centers for Disease Control, Atlanta, GA, U.S.A., Dept. of National Health and Population Development, Pretoria and National Institute of Virology, Johannesburg, South Africa

Congo-Crimean hemorrhagic fever (CCHF) virus is widespread in Africa, Eastern Europe, the Middle East and Asia. Cases have been recognized in South Africa since 1981. We studied 36 farms in the Northern Cape and Orange Free State; 9 case farms and 27 control farms, and collected sera and data on tick and animal exposure, farm techniques and personal practices, and sera from 1800 cattle, sheep and goats. Of 474 human sera 15 had IgG antibodies to CCHF virus (3.16%), nine of the 15 were recently identified cases, of which one died. Six (1.3%) with no history of illness compatible with CCHF had IgG antibody titers from 16 to 1024 to CCHF virus. Three of 64 (4.6%) asymptomatic case contacts had antibodies and 3/410 (0.73%) non-contacts. The relative risk was 6.30 confirming the importance of person-to-person spread. In 1986 there were 13 cases of CCHF per 100,000 population in the Kimberley region. The disease is sporadic but endemic, the risk of infection in persons in contact with a case appears significantly higher than the general risk of being infected by animal contact.

R3.32

MOLECULAR CLONING AND SEQUENCING OF THE S RNA OF K22 VIRUS, A HANTAVIRUS.....H.L. Regnery, C. Hang, M.P. Kiley, T. Sanchez, D.A. Auperin, and J.B. McCormick.....Centers for Disease Control, Atlanta, GA 30333, U.S.A.....

Hemorrhagic fever with renal syndrome (HFRS) has been recognized in various parts of the world in a variety of clinical forms. Epidemiological and serological data support the conclusion that the severity of disease depends at least in part on the infecting Hantavirus strain. Hantaan virus, 76-118, isolated from Apodemus agravius is the prototype virus associated with severe classical HFRS. K22 virus isolated from Rattus norvegicus has been identified as the causative agent of a moderate to mild form of the disease in China.

The antigenic relatedness and distinctiveness of various strains of Hantaviruses have been documented. To further understand the inherent differences on a genetic level, a cDNA library using K22 virus RNA as a template was prepared. The nucleotide sequence of the S genome RNA was determined from selected overlapping cDNA clones. The nucleotide sequence revealed similarities consistent with the published sequence of Hantaan virus S RNA (Schmaljohn et al., 1986. Virol. 155). Homologies of the nucleotide and amino acid sequences for the S genome RNA of K22 virus and Hantaan, 76-118, virus will be presented.

R3.33

PROTECTION OF RHESUS MONKEYS FROM A LETHAL LASSA VIRUS INFECTION BY VACCINATION WITH A VACCINIA-LASSA VIRUS GLYCOPROTEIN CONSTRUCT.....J.B. McCormick, S. Fisher-Hoch, D. Auperin, M. Castor, G. Perez, S. Kuo, S. Bauer, J. Esposito.....Centers for Disease Control, SPB, Atlanta, GA U.S.A.....

We have previously reported the protection of guinea pigs from a lethal Lassa virus infection by vaccination with a vaccinia virus (Wyeth strain) construct containing the glycoprotein gene of Lassa virus. We vaccinated six rhesus monkeys: two with 1.2×10^9 pfu of Lassa glycoprotein construct two with vaccinia virus (Wyeth strain) not containing the construct, and two with 3×10^4 $TCID_{50}$ of live Mopeia virus. The monkeys were monitored for clinical illness and an immune response. They were challenged 42 days after vaccination with 1×10^4 $TCID_{50}$ of Lassa virus. The monkeys which received the Lassa virus construct and Mopeia virus survived with only signs of mild febrile illness, and mild systemic abnormalities. These abnormalities were severe in the two fatally infected monkeys which had received the vaccinia virus. Viremia levels were 3 to 4 logs lower in the surviving monkeys compared to fatally infected ones. We conclude that the Lassa virus construct and Mopeia viruses can protect primates from a lethal infection with Lassa virus, however, the protection afforded may not be complete.

R3.34

ROLE OF T LYMPHOCYTE SUBSETS IN PROTECTION AND RECOVERY FROM HANTAAN VIRUS INFECTION IN MICE

K.YAMANISHI, H.Asada, M.Tamura, K.Kondo, H.Dohi, T.Nagai, T.Kurata, and M. Takahashi: Department of Virology, Research Institute for Microbial Disease, Osaka University, Suita, Osaka, Japan

Adult athymic nude (BALB/c backgroud) mice or inbred BALB/c mice were infected intraperitoneally with Hantaan virus (HV). Virus was isolated from various organs of BALB/c mice only for a short time after infection, but was isolated from various organs of nude mice consistently. Then, the effects of adoptive transfer of immune serum or immune T cells from BALB/c to nude mice before or after virus inoculation were examined. Before transfer, T cell fraction was treated with complement (C')(group 1), anti-L3T4+C'(group 2), anti-Lyt 1.2+C'(group 3) or anti-Lyt 2.2+C'(group 4). When transferred before virus inoculation to test effects on protection against infection, immune serum and T cells of groups 1,2 and 4 were effective. When transferred after HV inoculation to test effects on clearance of virus, group 1 was the most effective followed by group 2. These results suggest that humoral and cellular immunity both have roles in protection against HV infection, and that T cells possessing $L3T4^-$ $Lyt2^+$ marker on the cell-surface are especially important for elimination of infectious virus in vivo. The in vitro experiments of cytotoxic T cells will be discussed in the session.

R3.35

A G1 GLYCOPROTEIN EPITOPE OF LA CROSSE VIRUS IS A MAJOR DETERMINANT OF INFECTION OF AEDES TRISERIATUS MOSQUITOES. D. Sundin, USDA-ARS, Laramie, WY, USA; B. Beaty, Dept of Microbiology, Colorado State University, Fort Collins, CO, USA; N. Nathanson, F. Gonzalez-Scarano, Dept. of Microbiology, University of Pennsylvania, Philadelphia, PA, USA.

A variant (V22) of LaCrosse virus that was selected by growing the virus in the presence of a monoclonal antibody, was restricted in its ability to infect Aedes triseriatus mosquitoes, the natural vector of the virus. Only 15% of the mosquitoes that ingested V22 became infected and only 5% of these developed disseminated infections. In contrast, 89% of the mosquitoes that ingested parental LaCrosse virus became infected and 74% developed disseminated infections. When V22 was passed three times in Aedes triseriatus mosquitoes by feeding, a revertant virus, V22R3, was obtained that infected 86% of the mosquitoes ingesting this virus. In addition, V22R3 regained the antigenic phenotype and fusion capability of the parent LaCrosse virus. These results suggest that the specificity of LaCrosse - Aedes triseriatus interactions is markedly influenced by the efficiency of the fusion function of the G1 envelope glycoprotein operating at the midgut level in the arthropod vector.

R3.36

CRIMEAN-CONGO HEMORRHAGIC FEVER IN SOUTHERN AFRICA.

R. Swanepoel, A.J. Shepherd and P.A. Leman. National Institute for Virology, Sandringham 2131, Republic of South Africa.

From February 1981 to December 1986 42 cases of Crimean-Congo hemorrhagic fever (CCHF) were diagnosed in 29 outbreaks in various locations in South Africa. A further 2 cases which were imported, arose in Zaire and Tanzania while 3 cases were diagnosed in Namibia. 13 patients were infected by tickbite, 16 had contact with livestock tissues, 8 gained nosocomial infection and the remaining 10 merely resided in or visited a rural environment. Following a short incubation, patients suffered sudden onset of fever, nausea, prostrating headache and myalgia. Early leukopenia, thrombocytopenia and elevated serum transaminases were followed on days 3 to 5 of illness by a petechial rash and epistaxis, haematemesis and melena. The occurrence of 13/47 deaths indicates that the African disease is no less severe than that which occurs in Eurasia. Antibody to CCHF virus was detected in 2,460/8,667 (28%) cattle sera (140/180 herds) tested in South Africa, as well as in 347/763 (45%) cattle sera (32/34 herds) in Zimbabwe. It is inferred that the virus will yet be found to be widespread in all countries in Africa and Eurasia which lie within the limits of world distribution of ticks of the genus Hyalomma.

R3.37

AN EPIDEMIOLOGICAL STUDY ON HFRS IN ITALY. M.Balducci, P. Verani, L. Nicoletti, A. Renzi, M. Salvadori, M. Lombardi, F. Leoncini and D. Bartolozzi.
Laboratory of Virology, Istituto Superiore di Sanità, Rome; Dept.s of Nephrology and of Infectious Diseases, S.Maria Nuova Hospital, Florence; Epidemiological Investigation Unit for Viral Diseases, Toscana Region, Florence, Italy.

Some sporadic cases of Nephropatia (HFRS) occurred in Central Italy during the years 1985-86. General symptoms were more severe than in Nephropatia Epidemica of Scandinavia and milder than in Korean hemorragic fever. The serological diagnosis was done by means of indirect immunofluorescence antibody technique (IFAT) on aceton fixed Vero E6 cells infected with Hantaan and Puumala viruses. Antibody titers in sera of these patients did not give a clear evidence of infection with one of the two virus tested, suggesting the possible occurrence of related but as yet unidentified serotypes which circulate in Europe. Epidemiological data suggest that a focus of Hantaan-related virus could be present in a rural suburban area in the province of Florence. A serological survey was performed by IFAT on sera from wild rodents (Rattus norvegicus, Apodemus sylvaticus, A. flavicollis, Clethrionomys glareolus) trapped in different regions of Italy.

R3.38

TRANSPORT OF SEMLIKI FOREST VIRUS GLYCOPROTEINS IN UUKUNIEMI VIRUS-INFECTED BHK21 CELLS. H. Gahmberg, R.F. Pettersson, L. Kääriäinen. Recombinant DNA-Laboratory, University of Helsinki, Valimo tie 7, 00380 Helsinki, Finland.

A typical feature of cells infected with Uukuniemi virus (UUK) is that the glycoproteins G1 and G2 accumulate in the Golgi complex causing an expansion and vacuolization of this organelle. We have investigated whether a Golgi complex which is morphologically altered by UUK infection can carry out the glycosylation and transport to the plasma membrane of the Semliki Forest virus (SFV) glycoproteins in double-infected cells. The SFV glycoproteins were transported to the cell surface while UUK glycoproteins remained accumulated in the Golgi of double-infected cells stained for immunofluorescence microscopy. Using immunofluorescence, a cell surface radioimmunoassay, and pulse chase labeling followed by immunoprecipitation, endoglycosidase H treatment and SDS-PAGE analysis we could show that the transport of SFV glycoprotein E1 was retarded by about 20 min in double infected cells, the transport delay occurring between the ER and the middle Golgi.

E1 of SFV became sialylated in trans Golgi on its way to the plasma membrane as shown by surface labeling of double infected cells.

Our results indicate that the morphologically altered Golgi still is functionally intact, and capable of glycosylating and transporting proteins destined for the PM.

R3.39

WIDE NUCLEOTIDE DIVERGENCE IN ALL RNA SEGMENTS OF SNOWSHOE HARE VIRUS ISOLATES FROM MOSQUITOES. J.P.M. Clerx, B.J. Beaty and D.M. McLean, Dept. Molec. & Cell Biol., Univ. of Arizona, Tucson, AZ, Dept. Microbiol., Colorado St. Univ., Fort Collins, CO, (USA) and Division of Medical Microbiol., Univ. of British Columbia, Vancouver, B.C. V6T 1W5 (Canada)

We have analyzed similarities in nucleotide sequence of L, M, and S bunyavirus RNAs between prototype Snowshoe Hare virus (Montana, 1956) and isolates from larval and adult mosquitoes from Yukon Terr. (1975, 1976). T1-oligonucleotide analysis of S-RNAs indicated that the mosquito isolates were as different from each other as from the prototype virus. The number of nucleotide changes was estimated at 21 to 24 (2.1-2.4%). Significantly fewer changes were found in the overlap region of N and NS reading frames. Oligonucleotide comparison of 24-25% of the M-RNA sequences indicated even larger differences between prototype SSH and mosquito isolates: 150 and 154 nucleotide differences (3.3 and 3.4% of the total sequence). Similar analyses revealed approximately 240 nucleotide differences between the L-RNAs (3.5%) of these viruses.

R3.40

THE GENOMIC ORGANISATION OF TOMATO SPOTTED WILT VIRUS.
P.T. de Haan and D. Peters. Agricultural University, Department of Virology, Wageningen, The Netherlands.

Tomato spotted wilt virus (TSWV) is a structurally complex virus consisting of three nucleocapsids bounded by a lipid envelope. The virus contains either four or five proteins; the smallest protein being associated with the nucleic acid. Analysis of the virus after iodination or after treatment with bromelain suggests that two of the other proteins are located outside on the envelope. The genome consists of three RNA segments of mol. wt 2.7×10^6, 1.7×10^6 and 1.1×10^6. TSWV has been classified into a monotypic group on the basis of its structure, its nucleic acid and protein composition. RNA extracts of this virus are not infectious, but ribonucleoprotein preparations are. This suggests that the virus is negative-stranded. Virus specific proteins were detected after incubation of cell-free protein synthesizing extracts with TSWV-RNA. These results could not be confirmed and experiments demonstrate that the RNA has a negative polarity were not successful thus far.

Several similarities can be found between TSWV and some members of the Bynyaviridae. More data on the replication strategy and the intrinsic properties of the RNA segments are still needed before it can be considered as a definite member of this family. As a first results some sequence homology has been found between the first 18 bases at the 3' end of TSWV-RNA species and those at the 3' end of the RNA species of members of the five Bunyaviridae genera.

R3.41

MOLECULAR CLONING OF TOSCANA VIRUS (BUNYAVIRIDAE, PHLEBOVIRUS GENOME) C.Giorgi, L.Nicoletti, L.Accardi, P.Verani.
Laboratory of Virology, Istituto Superiore di Sanità, Roma, Italy.

Toscana virus is a member of the Phlebovirus genus of the family Bunyaviridae; it was isolated from Phlebotomus Perniciosus collected in Italy. This virus has been shown to be able to establish persistent infections both in vertebrate and invertebrate cells. To study the mechanisms involved in the viral replication both in persistent and lytic infections, the Toscana virus genome was randomly cloned by using RNA from the viral nucleocapsids and calf thymus DNA pentanucleotides as primers. The recombinant clones were probed with RNA from the different genome segments of the virus. The analysis of these clones will be presented.

R3.42

INHIBITORY EFFECTS OF CHINESE HERBS ALTERNANTHERA PHILOXEROIDES GRIBES ON THE REPLICATION OF HEMORRHAGIC FEVER WITH RENAL SYNDROME(HFRS) VIRUS IN VITRO. YANG ZaN-CHOU, B.L.ZHU, M.Y. ZHANG, VIRUS RESEARCH INSTITUTE, Hubei Medical College, Wuhan, China.

This paper reports inhibitory effects of alternathera philoxeroides Gribes against repiication of HFRS virus. Viral antigen can not be detected by ELISA and immunofluoresecence in infected cells and suspensions. Two of six different compounds of this vegetable drug can inhibit replication of HFRSvirus in Vero-B6 cell culture. Inhibitory concerntration is 78 ug/ml and 720ug/ml respectively. Results showed that inhibitory effects of drug against HFRS virus concerned with its compounds. It is considered that this drug is effective against HFRS virus. This is a new findings.

R3.43

DEVELOPMENT OF INACTIVATED CELL CULTURE VACCINE AGAINST HAEMORRHAGIC FEVER WITH RENAL SYNDROME (HFRS)

G. Song, Institute of Virology, Chinese Academy of Preventive Medicine, Beijing, China

After demonstration of high sensitivity of primary rat lung and hamster kidney cell cultures to the propagation of HFRS virus, a collaborative studies to develop formalin-inactivated cell culture vaccine against HFRS were carried out, at first in rat lung cell culture, and later on, in hamster kidney cell culture, and promising results were obtained: the virus titers in these cultures were 10^{8} or more, and the antigenic titers of the inactivated vaccines prepared in these two cell cultures were 1:256-1:512 by ELISA. The seroconversion rates of these vaccines in inoculated animals(rabbits, rats, mice, or hamsters) were nearly 100%,and antibody titers elicited in these animals ranged from 1:8@1:512 by ELISA, and arround 1:32-1:64 by neutralization test. A preliminary protection test of the hamster kidney cell vaccine (lot 85-2) performed in Meriones unguiculatus, showed a protection index of 100,000. Human trials will be made in this year.

R3.44

SEROLOGICAL EVIDENCE FOR HANTAAN (or RELATED) VIRUS IN ITALY

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Dept. of Tropical Diseases, 1st University of Rome, Italy; National Institutes of Health, Bethesda, Maryland, USA.

Indigenous human cases of Haemorrhagic Fever with Renal Syndrome (HFRS) have not yet been recognized in Italy, even though several cases have been reported from surrounding countries (France, Austria, Yugoslavia).

A serological study to determinate the prevalence of antibodies to Hantaan virus in randomly collected sera of residents in Central and North Italy (496 cases) using the indirect immunofluorescence test with Hantaan-virus strain 76/118 was conducted. An overall positivity (≥1:16) of 2.0% was observed, with the highest prevalence rate (6.25%) registered in a rural area north of Rome (Acquapendente). Titres ranged from 1:16 to 1:64. In the positive cases no medical history of haemorrhagic fever or renal disease has been recorded.

The prevalence rate of antibodies against Hantaan virus in Italy correlate well with those reported from other areas of Central and South Europe. These data show Hantaan or related viruses to be present in Italy and necessitate broader study of rodents and humans for a better understanding of the epidemiology of the disease in the country.

R3.45

VIRUS ISOLATION AND IMMUNE RESPONSE IN HUMANS INOCULATED WITH A LIVE-ATTENUATED JUNIN VIRUS (JV) VACCINE. J.G. Barrera Oro†, C.MacDonald, R. Kenyon, J. Meegan, F. Cole, Jr.,H.W. Lupton, and C.J. Peters. U.S. Army Medical Research Institute of Infectious Diseases and The Salk Institute, Government Services Division*, Fort Detrick, Frederick, Maryland 21701-5011, U.S.A.

Candid #1 (a live-attenuated JV vaccine against Argentine Hemorrhagic Fever) was given subcutaneously or intramuscularly to 24 men and 11 women over a dosage range of 2,000 to 58,000 PFU. Virus rescued by cocultivation was attempted serially from blood mononuclear cells (BMC) of all volunteers and from semen of 10 of them. Conventional virus isolation was also performed with throat swab, blood plasma, urine and spinal fluid of 10, 4, 4, and 1 vaccinees, respectively. Immune response to Candid #1 was measured by complement enhanced plaque reduction neutralization (C'PRN), ELISA, fluorescent antibody (FA) and lymphocyte transformation (LT).

JV was isolated only from BMC of 4 vaccinees in the second postvaccination week. JV immunity was detected by one or more tests in 34 of 35 volunteers (97%). No single test detected all JV immune individuals; 94% were detected by LT, 91% by ELISA, 88% by C'PRN, and 32% by FA. One volunteer with JV prevaccination immunity showed a secondary response by ELISA and FA after receiving Candid #1. The immune response persisted for at least 1 year in 3 of 3 vaccinees tested, as measured by LT, and in 2 of 3 vaccinees,as measured by C'PRN.

R3.46

CODING STRATEGY AND EXPRESSION OF THE M AND THE S GENOME SEGMENTS OF HANTAAN VIRUS. C. Schmaljohn. Virology Division, U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland, U.S.A.

Hantaan is the prototype virus of the proposed Hantavirus genus of Bunyaviridae. Both the medium (M) and small (S) RNA genome segments of Hantaan virus have been molecularly cloned and complete nucleotide sequences determined. The S segment consists of 1696 nucleotides and contains a single, open reading frame in the viral complementary sense RNA. Cell-free translation of RNA transcripts generated from full length cDNA demonstrated that the nucleocapsid protein was encoded by this gene. The M RNA segment, 3616 nucleotides, contained an open reading frame in the viral complementary sense which encoded both viral envelope glycoproteins, G1 and G2. Amino terminal sequence analysis of G1 and G2 revealed a gene order of 5'-G1-G2-3'. Carboxy termini of the envelope proteins were localized by immune-precipitation and Western blotting of Hantaan proteins with antisera to synthetic peptides corresponding to the deduced amino acid sequence of the polyprotein. In contrast to other members of the Bunyaviridae, no evidence of nonstructural coding regions could be detected in either the M or S genome segments of Hantaan virus.

Gene products of the M and S segments were expressed in a baculovirus system. Proteins generated were examined for antigenicity and immunogenicity.

R3.47

CHARACTERIZATION OF MONOCLONAL ANTIBODIES AGAINST TOSCANA VIRUS (BUNYAVIRIDAE, PHLEBOVIRUS). L. Nicoletti, M.G. Ciufolini, L. Bellisario, A. Marchi and P. Verani. Laboratory of Virology, Istituto Superiore di Sanità, Rome, Italy.

Several hybridoma cell lines that produce monoclonal antibodies against Toscana (TOS) virus structural proteins have been isolated by fusing spleen cells from TOS virus immunized BALB/c mice with the mouse myeloma cell line NSI/1. The initial screening of antibody-producer hybridomas was carried out with an enzyme-linked immunosorbant assay (ELISA). The antibodies were further characterized by radioimmunoprecipitation assay (RIPA) with ^{35}S-methionine-labeled antigens and by Western Blot. Biological properties of monoclonal antibodies were studied by neutralization, hemagglutination-inhibition (HI) and immunofluorescence (IFA) tests. The reactivity of monoclonal antibodies with different members of the Phlebovirus genus were studied by IFA and RIPA.

R3.48

EXPRESSION OF THE SRNA GENE PRODUCTS FROM SNOWSHOE HARE BUNYAVIRUS. D. A. Small, D. Bishop. NERC Institute of Virology, Mansfield Road, Oxford, OX1 3SR United Kingdom.

Snowshoe hare virus is a member of the california encephalitis serogroup and is composed of 3 single strandec negative sense RNAs designated S, M and L. The S viral complementary RNA codes for two gene products from overlapping reading frames; a nucleocapsid polypeptide N, and a nonstructural protein NSs.

The gene coding for N and NSs was isolated by restriction enzyme digestion and inserted into two different baculovirus vectors, derived from Autographa californica nuclear polyhedrosis virus. These vectors differed in the length of the 5' leader sequence upstream from the polyhedrin gene promoter and in one vector part of the 3' region had been deleted. The relative levels of expression were compared for the two vectors for N and NSs proteins. The NSs coding region of the SRNA was also expressed independently in both vectors with the objective of determining its biological function in infected cells and possible role in regulating the switch from transcription to replication in viral morphogenesis.

R3.49

INOCULATION OF ARGENTINE VOLUNTEERS WITH A LIVE-ATTENUATED JUNIN VIRUS VACCINE. J.I. Maiztegui, F. Feinsod*, A.M. Briggiler, C.J. Peters*, D.A. Enria, H.W. Lupton*, A.M. Ambrosio, E. Tiano, M.R. Feuillade, G. Gamboa, O. Conti, D. Vallejos, C. MacDonald*, and J.G. Barrera Oro*. Instituto Nacional de Estudios sobre Virosis Hemorragicas, 2000 Pergamino, Argentina, and *U.S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick, Frederick, Maryland 21701-5011, U.S.A.

Candid #1, a live-attenuated Junin virus vaccine candidate, was given to 14 volunteers from the endemic area of Argentine Hemorrhagic Fever. Four immune and 5 non-immune individuals received 51,000 PFU of Candid #1 and 5 non-immune individuals received placebo, intramuscularly. The non-immune volunteers were inoculated in double-blind fashion. No adverse clinical side effects attributable to the vaccine were observed in any of the volunteers during the first 7 postinoculation weeks. Routine hematological, blood chemistry, and urine tests remained within normal limits. Junin virus was isolated from blood mononuclear cells by co-cultivation from 1 non-immune vaccinee on the 9th postinoculation day. Rising titers of Junin virus antibodies were observed in 2 of 4 immune vaccinees 2 to 3 weeks after inoculation of Candid #1. Serologic conversion against Junin virus was detected 3 to 5 weeks after inoculation in 4 of 5 vaccinees. The results of this study were in complete agreement with those observed previously in American volunteers inoculated with Candid #1.

R4.1
A MODEL SYSTEM FOR THE IN VIVO DIAGNOSIS OF HERPES ENCEPHALITIS USING A RADIOLABELLED ANTIVIRAL DRUG AND EXTERNAL GAMMA SCANNING. G.M. Cleator, P.E. Klapper, J.M. Bruce. Departments of Medical Microbiology and Chemistry, University of Manchester, Manchester, U.K.

(E)-5-(2-carboxyvinyl)-2-deoxyuridine was radioiodinated with ^{125}I using a modification of the chloramine-T technique to produce (E)-5-(2-^{125}Iodovinyl)-2-deoxyuridine (^{125}IVDU). In in vitro studies the radio-iodinated metabolites of ^{125}IVDU selectively accumulated in virus infected cells.

Albino rats were infected with Herpesvirus simplex type 1 (strain SC-16) by a route known to induce a focal encephalitis. When neurological signs became apparent, the rats were anaesthetised and injected via the common carotid artery with Vepesid (Etoposide - Bristol-Myers, Slough, U.K.) to induce a temporary disruption of the blood brain barrier. ^{125}IVDU was then injected via the same route. The subsequent localisation of radioactivity in areas of viral infection was evaluated by external imaging using a gamma camera. The data obtained by gamma scanning was confirmed by sacrificing the animals and preparing cryostat sections of the brain. The distribution of viral antigens was related to the distribution of radiolabelled antibody by direct comparison of sections stained with peroxidase anti-peroxidase with sections prepared for autoradiography.

R4.2
A MODEL SYSTEM FOR THE IN VIVO DIAGNOSIS OF HERPES ENCEPHALITIS USING A RADIOLABELLED MONOCLONAL ANTIBODY AND EXTERNAL GAMMA SCANNING. P.E. Klapper, G.M. Cleator, H. Sharma, M. Longson. Departments of Medical Microbiology and Medical Biophysics, University of Manchester, Manchester, U.K.

Albino rats were infected with Herpesvirus simplex type 1 (strain SC-16) by a route known to induce encephalitis. When neurological signs became apparent, the rats were anaesthetised and injected via the common carotid artery with Vepesid (Etoposide) (Bristol-Myers, Slough, U.K.) to induce a temporary disruption of the blood brain barrier. Radiolabelled antibody (mouse monoclonal antibody to HSV glycoprotein D, labelled with ^{125}I) was then injected by the same route. The subsequent localisation of antibody in areas of viral infection was evaluated by external imaging using a gamma camera. The data obtained by gamma scanning was confirmed by sacrificing the animals and preparing cryostat sections of the brain. The distribution of viral antigens was related to the distribution of radiolabelled antibody by direct comparison of sections stained with peroxidase anti-peroxidase with sections prepared for autoradiography.

R4.3
ISOLATION OF HIV AND DETECTION OF ANTIBODIES IN THE CEREBROSPINAL FLUID OF PATIENTS WITH VARYING CLINICAL CONDITIONS.
E.M. Fenyö*, F. Chiodi*, G. Norkrans**, A. Sönnerborg***, E. Norrby*, B. Åsjö*. et al. *Department of Virology, Karolinska Institute, School of Medicine, Stockholm, Sweden, **Department of Infectious Diseases, Östra Hospital, University of Gothenburg, Sweden, ***Department of Infectious Diseases, Roslagstull Hospital, Karolinska Institute, Stockholm Sweden.

The acquired immunodeficiency syndrome (AIDS) is frequently complicated by central nervous system disfunctions and diffuse encephalopathy in which progressive dementia is the dominant feature.

We have attempted isolation of HIV from the cerebrospinal fluid (CSF) and blood from 30 patients with varying severity of HIV infection. Virus was successfully isolated from the CSF of patients in different clinical stages of HIV infection including asymptomatic carriers. CSF and serum antibodies to the virus were detected by ELISA and radioimmunoprecipitation assay (RIPA). All individuals with HIV-specific antibodies in serum also had antibodies in their CSF, regardless of the clinical stage of the infection.

The pattern of occurrence of antibodies to different viral proteins detected in the CSF, as compared to that in serum of infected patients, suggested a different processing of the HIV encoded proteins in the brain.

R4.4
QUATRE ANS DE SURVEILLANCE VIROLOGIQUE DES MENINGITES ASEPTIQUES A TEHERAN.
Kh. Farrohi, P. Maleknejad et H. Etemadi
Département de Microbiologie, Virologie et d'Immunologie de la Faculté de Médecine, Université de Téhéran. Téhéran- Iran
Durant quatre ans (de septembre 1982 à août 1986) nous avons virologiquement étudié un nombre total de 182 liquides céphalo-rachidiens, provenant d'enfants atteints d'une méningite dite aseptique. Parmi 182 L.C.R. obtenus, 23 s'accompagnaient avec ceux des selles et frottis de gorge, 15 avec matières fécales seules, et enfin 6 autres seulement avec le frottis de gorge du malade. En somme , durant cette étude, nos recherches virales ont porté sur 249 prélèvements dont 182 L.C.R. 38 matières fécales et 29 frottis de gorge.
A partir des liquides céphalo-rachidiens nous avons pu isoler 34 souches de virus soit un pourcentage global de 18%. Les virus isolés se repartissent de la façon suivante: une souche de virus poliomyélitique, 9 souches de virus coxsackie B, 8 souches de virus ECHO, 11 souches de virus des oreillons, une souche de virus hérpetique et enfin 4 souches de virus non identifiés. Aucun virus n'a pu être isolé à partir de 29 frottis de gorge étudiés. L'examen virologique de 38 matières fécales a révélé la présence de trois agents viraux dont les L.C.R. correspondant n'ont pas permis isolement d'un virus.

R4.5
Axonal transport of viruses
Erik Lycke, Bo Svennerholm, Krister Kristensson* and Henri Tsiang**, Dept Virol Univ Göteborg; Dept of Neuropathol K.I. (Huddinge Hosp)* and Unité Rage, Inst Pasteur**
The concept of a neuroganic transmission of virus infections by peripheral nerves to the central nervous system (CNS) and transfer of virus between different parts of the infected CNS by neural pathways has emerged from experimental and clinical observations. Thus some viruses e.g. rabies (RV) and certain herpesviridae such as the herpes simplex (HSV) and varicella-zoster viruses are transported by axonal flows in the retrograde as well as the anterograde direction. We have studied mechanisms for uptake and axonal transfer of RV and HSV in an in vitro system. Rat dorsal root ganglion neurons were cultivated in this system which allowed infection and manipulation of neuritic extensions without directly affecting the neuronal cell soma or vice versa. Attachment and uptake of the two viruses by neurites, the rates of their axonal transport and the mode of transport were examined. The main mechanism for internalization of HSV into neurites seemed to be fusion of viral envelope and neuritic plasma membrane. HSV nucleocapsids were transported axonally at a rate of about 50 mm per day whereas the rate for RV was estimated to 12 to 15 mm per day, suggesting that neurite-RV interactions might be of a more complex nature. Chemically induced arrest of axonal transport strongly indicated that transport of both the viruses was the result of microtubule-functions. Different mechanisms seemed operating for retrograde and anterograde transfer of virus.

R4.6
Primary and recurrent herpes simplex virus type 2 induced meningitis

Tomas Bergström, Anders Vahlne, Stig Jeansson, Kjell Alestig and Erik Lycke. Departments of Clinical Virology and Infectious Diseases, Guldhedsgatan 10 B, 413 46 Göteborg, Sweden.

A clinical study of 24 patients with meningitis caused by herpes simplex virus type 2 (HSV-2) is presented. Diagnosis was based on virus isolation from cerebrospinal fluid in 20 of the patients, and on virus isolation from genital lesions together with seroconversion against the HSV-2 type specific glycoprotein gG-2 in 4 patients. 15 of the 24 patients were female, and 20/24 were single. Mean age at onset was 25.7 years (range 16-40 years) and meningitis was preceeded by a genital lesion in 19 of the patients. Mean intervall between genital lesion and meningitis was 6 days (range 3-13 days). Cytological analysis of the cerebrospinal fluid showed a marked pleocytosis (mean 428 cells) with dominance of mononuclear cells. Spinal protein was moderately elevated, and spinal glucose was normal. Serological studies performed in 18 of the patients showed that 15 of these patients had a primary HSV infection, i.e. they had not antibodies to HSV-1 at the time of onset of meningitis. Antibody patterns shown by immunoblotting will be discussed. Focal neurological signs and symptoms were found in 8 patients: paresis of the urinary bladder (4), dysestesia (3), parestesia (2), neuralgia (2) and paresis (2), but were reversible in all patients. Relapses of meningitis occurred in four patients.

R4.7

ALPHA-INTERPERON RESPONSES IN CEREBROSPINAL FLUID OF PATIENTS WITH SUSPECTED MENINGITIS. D.O. Ho-Yen*, D. Carrington*†. Regional Virus Laboratory*, Ruchill Hospital and Virus Laboratory, Department of Microbiology†, Royal Hospital for Sick Children, Glasgow, Scotland.

Cerebrospinal fluid from 100 patients with clinically diagnosed meningitis was examined for α-interferon. In the laboratory four patient groups were identified: bacterial meningitis (n = 12), viral meningitis (n = 15), normal cerebrospinal fluid (n = 57) and abnormal cerebrospinal fluid (n = 16). A further 14 patients with cerebrospinal fluid shunts but no abnormality in the cerebrospinal fluid provided a control group for α-interferon determinations. The group with viral meningitis and the group with abnormal cerebrospinal fluid had significantly higher α-interferon concentrations ($p < 0.001$) when compared with those of the three other groups. This assay had great predictive value in determining those patients with abnormal cerebrospinal fluid who did not have a bacterial cause of meningitis. As the groups with abnormal cerebrospinal fluid and viral meningitis had a similar spread in α-interferon values it is likely that both reflect viral infection of the central nervous system.

R4.8

HTLV-I ASSOCIATED MYELOPATHY IN HOKKAIDO, NON-ENDEMIC AREA IN JAPAN. M.Shibata[1], T.Togashi[1], T.Matsuo[1], T.Chubachi[1], Y.Maruo[2], K.Tashiro[2]. Departments of Pediatrics[1] and Clinical Neurology[2], Hokkaido University School of Medicine, Sapporo, Japan.

HAM(HTLV-I associated myelopathy) is a relatively new disease entity, which was first described in Japan by Osame et al. in 1986, and is thought to be analogous to TSP (tropical spastic paraparesis). HAM or TSP is common in HTLV-I endemic areas or tropical areas respectively.

Hokkaido located in northern part of Japan has low prevalence(0.5%) of HTLV-I infection. We had screended 222 CSF samples for HTLV-I antibodies by particle agglutination method at Hokkaido University Hospital from May 1986 through December 1986 and found out 2 positive samples. One was from the patient with tension headache, and the other with slowly progressing spastic paraparesis. According to the result of the screening, the sera and CSFs of the patients with spastic paraparesis were examined. Eleven patients were found to have HTLV-I antibodies in both serum and CSF,they were 7 males and 4 females, whose ages ranged 30 years to 71. Three patients among 11 had histories of whole blood transfusion. None of them came from endemic areas, and they had neither leukemia nor lymphoma.

We concluded from the observasion that there were HAM even in non-endemic area and asymptomatic carriers in spite of the presence of CSF antibodies.

R4.9

T CELL RESPONSES IN THEILER'S VIRUS-INDUCED DEMYELINATING DISEASE. P. Borrow, C.J.R. Welsh, P. Tonks and A.A. Nash
Department of Pathology, University of Cambridge, Cambridge, U.K.

On infection with Theiler's murine encephalomyelitis virus (TMEV), susceptible mice develop a multiple sclerosis-like demyelinating disease. Susceptibility to demyelination is controlled in part by the H-2D locus, and current evidence suggests that both the immune system and the virus may participate in demyelination. However, the precise contribution of different mechanisms to pathogenesis is unclear.

T cell responses to TMEV have been compared in susceptible and resistant mouse strains and a direct correlation has been found between susceptibility to demyelination and the $L3T4^+$ T cell proliferative response to TMEV following injection of UV-irradiated virus in Freund's adjuvant. To further understand the roles T cell subsets play in pathogenesis/virus clearance, mice have been depleted of specific T cell subsets in vivo: results here suggest that $Lyt\text{-}2^+$ as well as $L3T4^+$ cells may be important in controlling virus replication.

To elucidate mechanisms by which T cells are activated within the CNS, in vitro cultures of neuroglial cells from different strains of mice have been established. It has been shown that TMEV is able to replicate in astrocytes and results from experiments on the ability of these cells to process/present viral antigens and interact with T cells in vitro will be discussed.

R4.10

PERSISTENTLY INFECTED NEUROBLASTOMA CELLS WITH MEASLES VIRUS (NS20Y/MS) EXPRESS MHC CLASS II GLYCOPROTEINS. J. Gopas, D. Izchaki, S. Segal and R. Rager-Zisman, Department of Microbiology and Immunology, Faculty of Health Sciences, Ben Gurion University of the Negev, Beer Sheva, Israel.

Measles virus is considered as a possible etiological agent for multiple sclerosis (MS), a chronic autoimmune demyelinating disease. To elucidate the mechanisms underlying demyelination, we investigated whether persistent measles virus infection of neuronal cells can trigger autoimmune reactivity. We used the persistently infected (PI) neuroblastoma line NS20Y/MS. Immunization of syngeneic A/J mice with NS20Y/MS elicited a strong CTL response against NS20Y/MS. These CTLs also recognized uninfected NS20Y cells. Cytotoxicity against NS20Y/MS was inhibited by anti measles or anti MHC class I antibodies indicating that the effector cells were virus specific class I restricted T cells. Furthermore, cytotoxicity was also inhibited by anti Ia^k suggesting that class II restricted CTL are generated as well. FACS analysis demonstrated that NS20Y/MS cells express increased levels of class I antigens, beta-2 microglobulin and Ia molecules on their cell surface. These observations suggest that persistent measles virus infection can induce the expression of immunoregulatory molecules, thus providing the obligatory basis for the development of autoreactive mechanisms in the CNS.

R4.11

A COMPARISON OF INTRA-BLOOD-BRAIN BARRIER (BBB) IgG SYNTHESIS IN PATIENTS WITH AIDS, ARC AND ASYMPTOMATIC HIV-POSITIVE, SSPE, AND NORMALS. P Shapshak, WW Tourtellotte, P Schmid, MA Osborne, L Resnick, G Rubinshtein, R Mitsuyasu, M Gottlieb. VA and UCLA Med. Centers, Los Angeles, CA.

Intra-BBB IgG synthesis occurs in viral infections of the Central Nervous System (CNS). Intra-BBB IgG synthesis was determined for HIV-seropositive patients with AIDS, ARC, or asymptomatic, SSPE patients, and normals by an elevated rate formula &/or the presence of CSF oligoclonal bands by isoelectric focusing (IEF). 80% (12/15) patients with AIDS Dementia Complex (ADC), 60% (3/5) patients with known CNS opportunistic infections, and 60% (3/5) neurologically asymptomatic patients had intra-BBB IgG synthesis. 93% (13/14) patients with SSPE had intra-BBB IgG synthesis. Only 7% (4/55) normals had elevated synthesis. Using an ultrasensitive ELISA to quantify virus-specific IgG in ng/ml in CSF and serum and applying these values to the rate equation, 5 of the above HIV-positive patients were found to have 10%, 7%, 3%, 0%, and 0%, respectively, of total IgG synthesis specific for HIV. SSPE is caused by measles and in 3 SSPE patients tested for comparison, 39%, 59%, and 82% of total intra-BBB IgG synthesis was measles-specific. The majority of HIV-seropositive patients, at all stages of disease and irrespective of neurologic disease, have an elevated intra-BBB IgG synthesis. Elevated synthesis may be indicative of a specific HIV CNS infection and may reflect of impending neurologic disease in neurologically asymptomatic patients.

R4.12

SEMLIKI FOREST VIRUS INDUCED, IMMUNE MEDIATED DEMYELINATION. J.K. FAZAKERLEY, S. Pathak, H.E. Webb. Neurovirology Unit, St Thomas' Hospital, London, UK.

Infection of adult mice with the avirulent A7(74) strain of the encephalitic alphavirus, Semliki Forest virus results in a transient viraemia, infection of the CNS, a pleocytosis, blood-brain barrier changes, abnormal VER's, and a demyelinating encephalomyelitis with involvement of the optic nerves. EM studies indicate preservation of the axons in the demyelinated lesions and demonstrate the close association of lymphoblastic cells, astrocytes and myelin. Immunosuppression experiments, and experiments in athymic 'nude', NK-cell deficient 'beige', and mice depleted of complement, indicate that the demyelination is immune mediated and directly dependant upon sensitised T-lymphocytes. It is not clear whether the immune mediated demyelination is a response to viral or autoantigens on the surface of the myelin or its supporting cells the oligodendrocytes. Athymic 'nude' mice develop a life-long infection of the CNS, and studies indicate that the virus also persists in normal immunocompetent adult mice, but cannot be detected by infectivity assay.

R4.13

HIV CORE ANTIGEN (Ag) IN THE CEREBROSPINAL FLUID (CSF) OF PATIENTS WITH AIDS. IZ Leiderman*,AR Flesher**,K Shriver**, BA Schaeffler**, F Spielberg**, MR Tam** and BR Adelsberg*. *Mt. Sinai School of Medicine, New York, NY and **Genetic Systems Corporation, Seattle, WA, USA.

Patients infected with HIV often develop central nervous system (CNS) disorders. We have studied 20 such patients, including 10 with encephalopathies, 8 with meningitis (4 cryptococcal, 4 viral), and 2 with polymyositis, for the presence of HIV p25 Ag in their CSF. To detect Ag, an assay was developed using monoclonal anti-p25 antibodies for capture and a polyclonal antiserum directly conjugated with HRP as signal. This assay is sensitive to at least 10 pg HIV/assay using a preparation of inactivated whole virus. Subsequent data analysis revealed that 10/10 patients with encephalopathies were scored as positive using our Ag capture assay, whereas 0/10 patients with meningitis or polymyositis were positive. The average OD value for the encephalopathic patients was 0.128 ± 0.097 compared with an average OD value of 0.017 ± 0.008 for the meningitis/polymyo- sitis group ($p<0.01$). In 10 of these cases where serum samples were also available, presence of Ag in CSF did not correlate with the presence of Ag in serum. These data support the conclusion that the AIDS dementia complex is related to direct HIV infection of the CNS, whereas meningitis and polymyositis are secondary features of AIDS.

R4.14

INTRATHECAL SYNTHESIS OF IgG OLIGOCLONAL BANDS WITH SPECIFIC ACTIVITY AGAINST HUMAN IMMUNODEFICIENCY VIRUS (HIV) IN AIDS PATIENTS WITH ENCEPHALOPATHY. M. Ceroni, G. Stone, P. Piccardo, D. Madden, J. Sever. NINCDS, NIH, Bethesda, MD.

Involvement of the nervous system, including encephalopathy, is increasingly recognized as a frequent finding in AIDS. Evidence is accumulating that the HIV virus not only attacks the immune system but also effects the central and peripheral nervous system. Our studies using isoelectric focusing of serum and CSF from patients with AIDS-related encephalopathy confirmed the presence of oligoclonal IgG banding. Some bands were common to serum and CSF and others present only in CSF. Using an immunoblot technique employing HIV precoated nitrocellulose paper, we have also demonstrated the presence of several HIV specific IgG bands in the sera and CSF of patients with AIDS-related encephalopathy. Most HIV antibody bands are present in both serum and CSF; some are present exclusively in the serum or CSF. Negative controls included sera from patients with gammopathy and HIV antibody positive samples blotted to nitrocellulose paper precoated with control, non-infected H-9 cell culture preparations. These findings demonstrate that HIV antibodies develop in the CNS and specific B-lymphocyte clones are stimulated to produce monoclonal IgG antibodies to HIV in the CNS. These findings strongly support a direct causative role of HIV in AIDS-related encephalopathy.

R4.15

CELL DIFFERENTIATION AND INFLUENZA VIRUS EXPRESSION IN MOUSE BRAIN CELL CULTURES. G.L. Bradshaw, R.W. Schlesinger, C.D. Schwartz. Dept. of Mol. Gen. and Microbiology, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ, USA 08854.

We are comparing viral gene expression, specifically of segments 5(NP), 7(M), and 8(NS), in 1^{o} and 2^{o} mouse embryo brain (MEB) cell cultures infected with "non-neurotropic" H1N1 strains A/PR/8/34 or A/WS/33 or the neurotropic derivative WSN, as a function of cell differentiation. We reported that (1) immature (6 day-old) MEB cultures produce single-cycle trypsin-activable PFU yields that are similar for all 3 viruses and also to yields from MDCK cells or CEF, (2) reduced yields of PR8 or WS in differentiated cultures consisting predom. of astrocytes are correlated with restricted M1 and NS1 (relative to NP) in astros, (3) restricted M1 and NS1 in astros is assoc. with intranuclear accum. of NP which can persist for >8 wks. Here, we show that (1) in contrast to astros, oligodendrocytes and neurons are unrestricted in NP, M1, and NS1 throughout cytoplasm and cell processes, (2) restricted M1 and NS1 are not accomp. by similar reductions of M2 and NS2 or by reduced 1^{o} transcription of the relevant gene segments, (3) PR8-infected, but not uninfected, astros display Ia MHC antigen. Current studies address the question of whether alterations in MHC Ag presentation are induced by abortively replicating virus itself or by IFN (which is present in infected MEB cultures). Implications for possible neuropathological manifestations induced by human influenza viruses will be discussed.

R4.16

HERPES SIMPLEX VIRUS TYPE 1 (HSV 1) INFECTION OF THE CENTRAL NERVOUS SYSTEM (CNS) IN MICE. L. Kastrukoff, A. Lau, S. Kim. Department of Medicine, Faculty of Medicine, University of British Columbia, Vancouver, Canada.

Focal demyelination in the CNS of mice has been observed following peripheral inoculation with HSV 1. To further define pathogenesis, studies of natural resistance to HSV 1 following lip inoculation were performed in 22 strains of mice and permitted their classification into resistant, moderately resistant, and susceptible groups. The pathological appearance of the CNS in a representative strain of each group, varied from focal collections of mononuclear cells in C57BL/6 mice (resistant) and unifocal demyelinating lesions at the trigeminal root entry zone (TREZ) in Balb/c (moderately resistant), to multifocal demyelinating lesions throughout the brain in A/J mice (susceptible).

Serial viral titration studies in the three strains implicate roles for both virus and immune mechanisms in the development of lesions at TREZ. Multifocal lesions in the brain appear to be the result of immune mechanisms alone but possibly triggered by the acute infection. Studies were undertaken to determine if differences in resistance to virus also exist at the level of the oligodendrocytes (MOL). Primary MOL cultures derived from 20 strains of mice were infected and examined serially using immunofluorescent, EM, and $TCID_{50}$ techniques. Differences observed in-vitro correlated closely with results of mortality and pathology and would support this as an additional mechanism in mediating resistance to HSV 1.

R4.17

AN ANALYSIS OF ANTIBODIES TO THE PARAMYXOVIRUS SV5 IN THE CSF's OF PATIENTS WITH MS. K.K.A. Goswami,[1] R.F. Randall,[1] L.S. Lange,[2] W.C. Russell[1]. Department of Biochemistry and Microbiology, University of St. Andrews, Fife, Scotland[1] and Department of Neurology, Charing Cross Hospital, London, England[2].

About 50% of multiple sclerosis patients appeared to have specific antibodies to the paramyxovirus SV5 in their cerebrospinal fluids whereas only 10% of neurological control patients had such antibodies and all of these had severe blood-brain barrier damage. In addition it was found that these SV5 antibodies constituted a significant proportion of the total CSF immunoglobulin in some MS patients and that the oligoclonal bands seen after isoelectric focussing of MS CSF's containing such specific SV5 antibodies could be absorbed out with a partially purified preparation of SV5 virus but not with measles virus.

R4.18

EXPRESSION OF MEASLES VIRUS PROTEINS IN INFECTED HAMSTER BRAINS IS AGE DEPENDENT. P. Swoveland and K. Johnson. Department of Neurology, Veterans Administration Medical Center and Univ. of Maryland, Baltimore, MD., USA.

Persistent central nervous system (CNS) infection with measles virus results in a fatal encephalitis of children and young adults called subacute sclerosing panencephalitis (SSPE). Age at infection has been shown to be a major determinant in the development of persistent CNS disease in humans and in a hamster model of SSPE. Hamsters infected at various ages have been studied to determine the effect of CNS maturation on expression of measles virus.

Animals were infected with the HBS strain of virus at intervals between 1 and 21 days of age. Brains were examined 5 days later for viral titers, immunocytochemical localization of individual viral proteins, and Western blot analysis of viral proteins. Serum titers of anti-measles IgG were determined by ELISA. Anti-measles IgG was not detectable in any animals inoculated at 15 days or less, but titers of cell-free virus were progressively lower with increasing age. Between days 10 and 15, expression of H protein decreased dramatically in neurons and oligodendrocytes, while NP and M remained at high levels. These results suggest that developmental status of CNS cells affects expression of measles virus proteins which in turn may determine lytic or persistent infection.

R4.19

SEMLIKI FOREST VIRUS INFECTION AND SUSCEPTIBILITY TO Experimental Allergic Encephalomyelitis. Foroozan Mokhtarian. Dept. of Neurology, School of Medicine, University of Maryland at Baltimore. Baltimore, MD 21201

Six to 8 week old B6 mice, inoculated intraperitoneally with the A774 strain of Semliki Forest Virus (SFV), usually developed a mild to moderate subclinical encephalitis with virus levels peaking on day 6 and disappearing by about day 10 postinfection. A transient demyelination was seen at 2 weeks after SFV infection. Following recovery from this infection, B6 mice, normally resistant to the induction of experimental allergic encephalomyelitis (EAE), now became susceptible to it. SFV-recovered mice, but not normal B6 mice, developed signs of EAE following their inoculation with Myelin basic protein (MBP) in CFA. The SFV-infected mice, responded to MBP in blast transformation assay mostly during the first week after infection. This coincided with the presence of viral antigen in endothelial cells of brain. It is concluded that the activation of endothelial cells is antigen may result in presentation of MBP to the immune system which may be the mechanism for late demyelination and predisposition to EAE-induction. Supported by USPHS/NIH Grant NS-20022 and RG-1904-A-1 from the National Multiple Sclerosis Society.

R4.20

MOUSE HEPATITIS VIRUS RECEPTOR MAPS TO THE CHROMOSOME 7 SITE REGULATING RESISTANCE TO VIRUS REPLICATION. R.L. Knobler, D.G. Weismiller, R.K. Williams, C. Cardellichio, K.V. Holmes. Jefferson Medical College, Philadelphia, PA, Uniformed Services University, Bethesda, MD, USA.

Previous studies in recombinant-inbred (RI) mice demonstrated linkage of the gene regulating resistance to fatal disease induced by mouse hepatitis virus type 4 (MHV-4, strain JHM) to the Svp-2 locus on mouse chromosome 7. This locus, named Hv-2, also regulates replication of a closely related coronavirus, MHV-A59. Recently, it has been demonstrated that resistance to MHV-A59 replication, in SJL mice, correlates with absence of a receptor for the virus. This 110K glycoprotein mouse hepatitis virus (MHV) receptor is demonstrated on cell membranes from susceptible BALB mice, with a virus overlay protein blot assay (VOPBA), or a monoclonal antibody directed against this MHV receptor. In the present analysis, RI strains, characterized as susceptible or resistant to MHV-A59 and MHV-4, were tested for presence of the receptor. Only those RI strains susceptible to these two viruses had detectable virus receptor by VOPBA or antibody binding. Resistant strains did not have the receptor. These data demonstrate that resistance to replication of these two viruses correlates with absence of a virus receptor, which may be the structural gene product of the gene regulating resistance to virus replication mapped to mouse chromosome 7.

R4.21

EFFECT OF RAV-7 ON THE AVIAN CENTRAL NERVOUS SYSTEM

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Infection of chickens in ovo with RAV-7 leads to obesity and stunting, accompanied by lymphocyte infiltration of the thyroid gland, and other indications of lymphoproliferation. Severely affected chickens manifest symptoms of neurologic dysfunction including ataxia, stupor, circling, and hyperreflexes. Measurements of peripheral nerve conduction velocity showed that nerves of infected and uninfected chickens transmitted compound action potentials at 65 m/sec. Measurements of ascending evoked spinal cord potentials showed that RAV-7 infected chickens had conduction velocities of 62.5 ± 16.3 m/sec and uninfected age-matched chickens 104.8 ± 13.1 m/sec. Examination of obese hypothroid chickens (OS) and age-matched controls (Special C) revealed that the conduction velocities of hypothroid and euthroid chickens were identical The lesion in the brain and spinal cord of the RAV-7 infected chickens was characterized as a subacute, nonsupperative encephalomyelitis with perivascular accumulation of lymphoid cells and macrophages. There was little evidence for structural alteration of nervous parenchyma including neurons, but occasionally astrocytic hypertrophy and hyperplasia were present.

R4.22

TUBULOVESICULAR STRUCTURES IN NEGATIVELY-STAINED PREPARATIONS OF SCRAPIE. H.K. Narang, D.M. Asher, and D.C. Gajdusek. Public Health Laboratory, Newcastle General Hospital, Newcastle upon Tyne, England and National Institutes of Health, Bethesda, Maryland, USA.

Unique tubulovesicular particles of average diameter 23 nm have been consistently observed by electron microscopy (EM) in in thin sections prepared from the brains of animals and humans with spongiform encephalopathies, where they occur most often in postsynaptic terminals. The tubulovesicular structures are the only virus-like particles to be so regularly detected in the spongiform encephalopathies, but their relationship, if any, to the etiologic agents of the diseases remains unknown. It is important that the particles be concentrated and identified by negative staining in order to perform inmunoEM investigation, biochemical analysis, and to establish any relationship with infectivity. After repeated attempts to find the structures by conventional negative staining with suspensions of infected tissue failed, we used a "touch" or "impression" technique in which moist EM grids were laid on the cut surface of infected brain, rinsed, and then stained with ruthenium red and phosphotungstic acid. That method gave satisfactory demonstration of known virus particles in preliminary studies with experimental picornavirus, herpesvirus and flavivirus encephalitides in mice, and in human herpes encephalitis. Brains of hamsters infected with the 263K strain of scrapie agent were examined at various stages of illness, and negatively-stained impression preparations were compared with thin sections. In the impression preparations we found large aggregates of tubulovesicular structures generally resembling the tubulovesicular particles seen in thin sections, differing in that their average diameter was 30 nm. The aggregates were most often observed in fields containing larger vesicles of 50 nm diameter, probably synaptic vesicles, that were also found in normal brains. Further attempts to find the structures in impression grids prepared from other spongiform encephalopathies and in a variety of negatively-stained tissue extracts are in progress.

R4.23

EFFECTS OF PERSISTENT MUMPS VIRUS INFECTION ON THE EXCITABILITY OF PC12 AND TE671 CELLS. R.J. Ziegler and E.K. Stauffer. Depts. of Microbiology and Physiology, University of Minnesota-Duluth, School of Medicine, Duluth, MN 55812.

The effects of mumps virus on the morphology and physiology of two excitable cell lines were studied. Persistent mumps infections were established in rat pheochromocytoma (PC12) and human medulloblastoma (TE-671) continuous cell lines. Significant amounts of infectious virus were produced by the PC-12 cells, while infectious virus production by the TE-671 cells was limited. This restricted replication may be due to decreased production of viral envelope glycoproteins by the TE-671 cells. Neither the doubling time nor the morphology of either cell line was affected by the presence of the virus. Viral presence changed the distribution of stimulus-evoked electrical responsiveness of both PC-12 and TE-671 cells from a responsiveness composed primarily of normal rapidly-rising, all-or-none action potentials to one dominated by abnormal slow-rising, graded responses or by no response at all. Such changes has the potential to disrupt neural integration within the nervous system, and suggests a mechanism by which persistent mumps and other paramyxovirus infections play a role in chronic neurologic and/or mental disease.

R4.24

MENINGITIS DUE TO TOSCANA VIRUS IN ITALY:A TEN YEAR SURVEY. M. Balducci,P.Verani,M.G.Ciufolini,S.Caciolli,A.Renzi,L.Nicoletti P.Paci,F.Leoncini,G.Volpi,L.Amaducci, P.Padovani and E.Traini. Lab.of Virology,Istituto Superiore di Sanità,Rome;Dept. of Infectious Diseases,S.Maria Nuova Hospital,Florence;Dept. of Neurology,University of Florence;Dept. of Infectious Diseases Hospital of Fermo; Epidemiological Investigation Unit for Viral Diseases,Toscana Region,Florence,Italy.

Toscana Virus(family Bunyaviridae,genus Phlebovirus) has been repeatedly isolated since 1971 from both Phlebotomus perniciosus and P.perfiliewi sand flies in some rural areas of Central Italy. In the period 1977-1986,more than 100 cases of meningitis occurring during summer in two regions of Central Italy(Tuscany and Marche)were serologically diagnosed as cases of Toscana virus infection. For the Tuscany region,Toscana virus meningitis represented respectively 50% or 20% of viral CNS infections hospitalized during summer or the whole year. All patients were studied for their clinical course and for sequelae. The etiological diagnosis was obtained by showing seroconversion and/or presence of specific IgM antibodies. Furthermore,Toscana virus was isolated from cerebrospinal fluids of a few patients. All cases occurred among people living in suburban or rural areas where Toscana virus foci have been often demonstrated.

R4.25
IgG SUBCLASS ANTIBODIES AGAINST HIV AND CMV IN CSF AND SERUM FROM HIV INFECTED PATIENTS. T. Mathiesen[a,b], A. Sönnerborg[c,d], M. von Sydow[d], and B. Wahren[a], Dept. Virol., National Bacteriological Laboratory[a], S-105 21 Stockholm, Sweden; Dept. Neurosurg., Karolinska Hospital[b], S-104 01 Stockholm, Sweden; Dept. Infec. Dis., Roslagstull Hospital[c], S-114 89 Stockholm, Sweden; Central Microbiol. Laboratory of Stockholm County Council[d], S-101 22 Stockholm, Sweden.

Cerebrospinal fluid (CSF) and serum samples from 17 patients seropositive for Human Immunodeficiency Virus (HIV) were studied by ELISA for specific IgG1-4 against HIV and cytomegalovirus (CMV). All patients had IgG1 antibodies against HIV in both CSF and serum, and all had CMV IgG1 in serum, 16 in CSF. Anti HIV IgG was synthesized intrathecally in nine patients, four of which showed neurological symtoms. Intrathecally produced HIV IgG was most often of the IgG1 subclass. Six patients had evidence of intrathecal CMV antibody production. Intrathecally synthesized IgG3 reacting with HIV was found in two patients and IgG2 or IgG3 reacting with CMV in two more.

The expression of HIV-specific IgG subclasses in CSF and serum was more restricted in AIDS patients than in HIV infected persons without an AIDS diagnosis. The largest number of CMV-specific IgG subclasses was found in AIDS patients. Analysis of all IgG subclasses allowed intrathecal IgG production to be detected in more patients than assays or total IgG.

R4.26
NEUROINVASIVENESS OF WEST NILE VIRUS (WNV) STRAINS AND THE USE OF MODULATORS OF THE BLOOD BRAIN BARRIER (BBB). Yair Akov, Menachem Halevy, Shlomo Lustig, Israel Institute for Biological Research, Ness-Ziona, Israel.
The mode of penetration into the central nervous system (CNS) of mice, of a flavivirus, West Nile Virus (WNV) was studied with the aid of a variant, WN-25 which lost its neuroinvasiveness but not its neurovirulence. Intravenous (IV) injection of 10^6 PFU of either virus did not change the course of infection. Use of BBB modulators (glycerol, CO_2, DMSO) changed the disease in IV injected mice to resemble that obtained in mice-injected ic. Clearance rates of both viruses from the blood were similar as was the time of appearance of antibody. However, with WNV there was low viremia and penetration into the CNS, not present with WN-25. It is proposed that WNV after primary peripheral replication causes a low viremia and concomitantly causes a brief breach of the BBB, allowing WNV into the CNS. WN-25, though it replicates slightly, lacks these abilities. Injection of WN-25 IV into mice may serve to test substances for their ability to breach the BBB.

R4.27
IN SITU HYBRIDIZATION STUDIES IN INFLAMMATORY CASES OF MULTIPLE SCLEROSIS, WITH MEASLES VIRUS SPECIFIC RNA PROBES. S.L. Cosby[1,2], M. Bailey[1], M.J. Taylor[2], I.V. Allen[2], B.K. Rima[2], S.J. Martin[1]. Departments of Pathology (Neuropathology)[1] and Biochemistry[2], The Queen's University of Belfast, U.K.

Subacute sclerosing panencephalitis (SSPE is an inflammatory disease of the central nervous system (CNS), associated with persistent measles virus infection. One category of Multiple Sclerosis (MS) has also been recognised in which the degree and extent of perivascular inflammation within the CNS is disportionally greater than the degree or phase of demyelination. The significance of the widespread inflammation is uncertain but may be a key to the pathogenesis of MS. In this in situ hybridization study single stranded RNA probes have been used to examine inflammatory cases of MS for measles virus gene expression and compared with results for SSPE tissue. Positive hybridization has been obtained in MS brain tissue with a probe specific for the N gene sequence of the genomic RNA of measles virus. Preliminary results with probes to other measles virus gene sequences also indicate the specificity of the reaction for measles virus RNA. Inflammatory cells (including those in perivascular cuffs), in addition to several types of neural cells exhibit hybridization in both SSPE and MS brain tissue. However, hybridization in MS brain is localised to discrete foci and occurs at lower levels than in SSPE brain.

R4.28
ROLE OF CELLULAR IMMUNE FACTORS IN CORONAVIRUS A59 INDUCED DEMYELINATION, M.J.M. Koolen* and M.J. Buchmeier.
Dept. of Immunology, Scripps Clinic and Research Foundation, La Jolla, U.S.A., *Present address: Central Lab. Netherl.Red Cross Blood Transfusion Service, incorporating the Lab. of Exp. and Clin. Immunology of the Univ. of Amsterdam, Amsterdam, The Netherlands

Cellular immune factors involved in mouse hepatitis virus (MHV) strain A59 and a temperature-sensitive mutant (ts-342)-induced demyelination were studied in normal and athymic nu/nu BALB/c mice as well as in mice depleted of specific subsets of T lymphocytes in vivo. Intracerebral inoculation of normal BALB/c mice with 10*5 PFU of ts-342 resulted in prolonged infection of the central nervous system, whereas 100 PFU of the wild type virus were lethal. In athymic nu/nu mice, both wild type virus and ts-342 caused a fatal hepatitis suggesting that cellular immune factors are involved in the protection of mice against lethal MHV-infection.

Furthermore, significant levels of proliferation, measured as ^{3}H-thymidine incorporation, were observed when splenocytes isolated from ts-342 infected normal mice were cultured in the presence of either viral antigen or myelin basic protein (MBP). The responder cells were shown to be T lymphocytes, and in vivo depletion of the L3T4 population reduced the proliferative response to MBP to baseline levels. Consequently, we studied the significance of these MBP-specific T lymphocytes as possible autoimmune mediators in virus-induced demyelinating disease.

R4.29
IN VIVO AND IN VITRO STUDIES OF BORDER DISEASE VIRUS IN SHEEP. B. Potts, G. Elder, J. Sever, M. Sawyer*, B. Osburn*. Infectious Diseases Branch, IRP, NINCDS, NIH, Bethesda, MD and *Department of Pathology, School of Veterinary Medicine, University of California, Davis, CA, USA

Border disease (BD) is a congenital condition of sheep caused by a virus in the family Togaviridae genus Pestivirus. Affected lambs have reduced central nervous system (CNS) myelin, remain persistently infected and develop no viral neutralizing antibody. Dual immunocytochemical labeling for viral and cell type specific antigens was used to study in vitro and in vivo the type of neural cell infected and the virus induced disruption of cell functions. In the newborn ovine CNS approximately 1% of astrocytes, 10% of myelin basic protein (MBP) positive oligodendrocytes and up to 50% of cerebellar granule cell neurons contained viral antigen. Associated with this viral persistence was a decrease in three CNS myelin proteins. In fetal ovine CNS, BD viral antigen was again detected in 1% of astrocytes but other infected cells could not be identified because the differentiation antigens that identify mature neurons and oligodendrocytes were negligible. The in vitro studies using primary cultures derived from fetal and adult ovine CNS revealed that all major cell types, including oligodendrocytes, astrocytes, neurons and precursor cells were infected with BD virus. These data suggest that although the virus infects many cell types in the CNS, it affects the differentiated functions of oligodendrocytes at a critical developmental stage causing a significant delay in myelinogenesis.

R4.30
INFLUENCE OF CEREBELLAR DIFFERENTIATION ON THE TRANSCRIPTION OF JC VIRUS EARLY REGION. M. Matsuda, K. Yasui.
Department of Microbiology, Tokyo Metropolitan Institute for Neurosciences, Tokyo, Japan.

The relationship between the cerebellar differentiation and the transcription of JC virus early region was elucidated ,in vivo. Newborn hamsters were inoculated with JC virus Tokyo-1 strain, and consecutively JC virus T antigen mRNA was detected by in situ hybridization method.

mRNA of JC virus T antigen was not detected in the cerebellum until the tenth day, when several cells both in the internal granular layer and in the molecular, but not in the immature external granular layer, were found to contain JC virus T antigen mRNA. On the fifteenth day, the amount of T antigen mRNA decreased, and could not be detected on the twentieth day. On the thirtieth day, much T antigen mRNA was found on a small cell nest in the internal granular layer, which was considered as a medulloblastoma in situ. These results revealed that the cerebellar granular cell acquired the capability of transcribing the JC virus early region through its differentiation from external granular cells to internal granular cells.

R4.31

CHARACTERIZATION OF VIRAL RNAs IN RABIES INFECTED CULTURED NEURONS AND IN THE CNS. A. ERMINE, D. VEIGA-SACRAMENTO, P.E. CECCALDI, N. TORDO, H. TSIANG. Institut Pasteur, Unité Rage, 25, rue du Docteur Roux 75724 PARIS Cédex 15,France.

Kinetics of rabies RNA synthesis of cultured human neuroblastoma (IMR-32) and brain regions are investigated in both fixed and street rabies viruses acute infections. Street rabies virus persistently infected (IMR-32) is also studied. The total RNA are extracted either from infected cultures or from dissected brain areas. The RNA are run in agarose gel, andblotted on nylon membranes. Specific rabies RNA are detected after hybridization with 32-P labelled cDNA probes. Digital imaging method is used for standardization of each sample and to tentatively quantify the results.

The results from these studies allowed characterization of the specific viral RNA in the infected structures. The differential features of each case are discussed with regard to the naturally occurring disease.

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R4.32

THE STUDY OF ANTIBODIES TO PERIPHERAL NERVE TISSUE AND ANTIGENIC COMPONENTS IN PATIENTS WITH GUILLAIN-BARRE SYNDROME AND ITS SIGNIFICANCE IN CLINICAL APPLICATION

Bao-Lin Li,Hui-Lian Wu,Wei-Ping Wu,Feng-zhu chang, Fan Wu,Department of microbiology,Institute of Basic Medical Sciences,Shandong Academy of Medical Sciences Jinan,China.

Complement-fixing antigen components to the sera ofGuillain-Barre Syndrome (GBS)patients have been extracted from human nerve tissues by chloroform-methanol-H_2O system. This antigen has been found to be composed of 2-4 compoents,which were demonstrated by immunodifrusin test,immunoelectroosmophoresis,rocket immunoelectrophoresis and crossed immunoelectrophoresis.

Of 81 subjects with GBS,74.1% had circulating complementfixing antibodies to the antigen; only 7.1%-17.6% was found to be positive in the sera of 71 subjects with other diseases; and negative in the sera of all normal subjects.

The methods of antigen preparation and the technique of complement fixation test used in the present work may be applied in clinical diagnosis and immunological study of GBS.

R4.33

INTRATHECAL SYNTHESIS OF IgG SUBCLASS IN HERPES SIMPLEX ENCEPHALITIS (HSE). T. Morishima, J. Yoshida, T. Osuga, H. Nagai, M. Miyazu, N. Hanada and H. Naruse. Department of Pediatrics, School of Medicine, Nagoya Univ. Nagoya, Japan.

In order to determine the kinetics of anti-HSV IgG subclasses produced in the central nervous system (CNS), CSF and sera were collected from 6 patients of HSE. The antibody activities against HSV were examined by ELISA. Purified HF strain of HSV-1 was used for antigen coating of microplate. Diluted CSF (X5) or sera (X100), mouse monoclonal antibodies to human IgG1-4, and peroxidase-conjugated anti-mouse goat IgG were added to the plate in sequence.

In sera of HSE, IgG1-3 activities were detectable on day 10 of the illness and increased in titer gradually, while IgG4 activity increased later. In contrast, IgG1 activity swiftly increased first in CSF, followed by the elevation of IgG3. IgG2 and IgG4 in CSF remained lower during the illness, while high titer of IgG1 continued in the convalescence. In severe and prolonged cases of HSE, IgG3 activities elevated and prolonged longer than in mild form of HSE. In order to neglect the effect of serum influx through the damaged blood-brain barrier, anti-HSV IgG1-4 in CSF and sera were assayed in other CNS viral infections, such as mumps meningitis. No significant difference was seen in IgG1-4 titer between CSF and sera. Thus, the regulation of local IgG1-4 synthesis may be related to the immunopathological changes of CNS in herpes simplex encephalitis.

R4.34

INHIBITION OF VIRAL TRANSPORT IN THE BRAIN. P.E. Ceccaldi, J.P. Gillet, H. Tsiang. Institut Pasteur, Département de Virologie, Unité Rage, 25, rue du Docteur Roux, 75724 Paris Cédex 15, France.

We have shown the retrograde transport of rabies virus in the rat brain, by inoculating the virus stereotaxically, and monitoring the presence of virus in anatomically connected brain areas by fluorescence studies (J. Neuroth. Exp. Neurol 1986, 45, 619-634). Infected neurons were observed in the cortex, substantia nigra, thalamus and the hippocampus in the ipsilateral brain showing a retrograde transport of rabies virus in the axoplasmic flow. Here, we interrupted the viral spread by stereotaxic inoculation of colchicine at the site of virus delivery.. Both fixed rabies and street rabies viruses were inoculated simultaneously or after the colchicine treatment. The observation of frozen sections after specific fluorescence staining shows that: 1/ the kinetics of viral spread is modified; 2/ some brain areas are not infected in the ipsilateral side with respect to the site of colchicine injection. Mainly the neostriatum, the cortex and the hippocampus characteristics of infection are modified by colchicine. These results are arguments that it is possible to interrupt the viral transport of virus from one brain area to another.

(This investigation was supported by grants from MRI N° 82.L. 1107 and INSERM, CRE N° 846019).

R4.35

AN EPIDEMIC OF WEST NILE VIRUS MENINGOENCEPHALITIS. M.S. Smith, W.H. Pieterse. Department of Medical Microbiology (Virology), Faculty of Medicine, University of the Orange Free State, Bloemfontein, South Africa.

An epidemic of meningoencephalitis involving almost 5 % of the population of the town of Wolmaransstad in the South-Western Transvaal, South Africa, occurred during 1984. The aetiological agent was serologically implicated as being West Nile (WN) virus. The peak of the epidemic coincided with the period of highest rainfall, 147 cases were recorded in a single general pratice, and 53,7 % of the patients were in the age group 11 - 20 years. The classical symptoms of WN fever were virtually absent and most patients had an uneventful recovery, although in several patients recovery was prolonged.

This is the first reported epidemic of WN meningoencephalitis to have occurred worldwide: previously reported cases have occurred sporadically.

R4.36

INTRATHECAL SYNTHESIS OF ALPHA INTERFERON IN NEWBORN AND INFANTS WITH PROGRESSIVE FAMILIAL ENCEPHALOPATHIES. P. Lebon, J. Badoual, G. Ponsot, F. Goutières, F. Cukier, J. Alcardi. Institut National de la Santé et de la Recherche Médicale, Unité 43, Hôpital St. Vincent de Paul, 74 av. Denfert-Rochereau, 75674 Paris Cedex 14, Paris, France.

Seven infants from six families who developed a progressive disorder of the central nervous system with calcifications of the basal ganglia and white matter alterations were investigated.

In two cases, alpha interferon was detected in sera and CSF at different times between birth and five years. In four other cases, a single sampling at either one, two or five years revealed its presence. Comparatively, IFN was not found in 44 infants with other neurological encephalopathies. In these familial encephalopathies, IFN is synthesized mainly in CSF associated with a lymphocytosis. No gamma interferon was detected at the same time in the same sampling of sera or CSF. The role of alpha interferon in the genesis of these calcifications and encephalopathies is discussed.

These results could be in favour of the existence of a chronic viral disease with familial predisposition. The serologic tests and cultures exclude infections with HIV-I and II, HTLV-I, rubella, CMV, VZV, toxoplasmosis and chlamydia in these children.

R4.37

SEQUENCE ANALYSIS OF THE NEUROVIRULENT DETERMINANTS IN THE GENOME OF ts1, A PARALYTOGENIC MUTANT OF MoMuLV. Wong, P.K.Y P. Szurek, T.Tzeng and P.H. Yuen. The University of Texas System Cancer Center, Science Park-Research Division, Smithville, Texas.

To better understand the molecular mechanism involved in MuLV induced paralysis in mice, recombinant viruses were constructed between the paralytogenic mutant, ts1 and the non paralytogenic wild-type parents MoMuLV and MoMuLV-TB. Two domains in the genome of ts1 were found to be responsible for its paralytogenic ability. One of these domains which determines the inability of ts1 to process Pr80^{env} extends from the XbaI site at nt 5765 to the BamHI site at nt 6537. This fragment encodes the 5' half of gp70. The other domain determines the enhanced neurotropism of ts1 including the 5' half of gp70, p15E and LTR. Sequence analysis of these two domains reveals that three base substitutions in the XbaI-BamHI region of ts1 at nt 5948, 5958, and 6344 resulted in amino acid substitutions. However, the change at n5 5958 also occurs in MoMuLV-TB. In the BamHI-ClaI regions, there are six base substitutions in ts1 which result in six amino acid changes. However, only the substitution at nt 7164 and nt 7250 are unique to the ts1 genome. The correlation of these alterations to the neurovirulence of ts1 will be discussed.

R4.38

INVOLVEMENT OF GANGLIOSIDES IN THE CELLULAR RECEPTOR-COMPLEX TO RABIES VIRUS. F. Superti*, C. Conti*, B. Hauttecoeur°, M.J. Morelec°, H. Tsiang°. * Institute of Microbiology, University "La Sapienza" Rome, Italy; ° Pasteur Institute, 25, Rue du Docteur Roux, 75724 Paris Cédex 15, France.

We have shown previously that CER fibroblasts treated with neuraminidase loose their capacity to be infected by rabies virus during 1-4 hours, thus advocating for the participation of cellular sialic residues as a possible component of a viral receptor-complex structure. These desialylated cells recover their susceptibility to rabies virus infection when preincubated with purified ganglioside mixtures immediately after the enzyme treatment. The use of individually purified gangliosides showed the involvement of highly sialylated gangliosides (GT1b, GQ1b) whereas monosialylated gangliosides were not active.

Solubilized rat brain membranes were active and compete with rabies virus infection of both CER and murine neuroblastoma (N115) cells. Extracted components from these membranes were also used to compete with viral infection. Here again, gangliosides were the most active components in both CER and N115 cells. These data are arguments that ubiquitary gangliosides may be involved in early steps of rabies virus infection. [illegible]

R4.39

HUMAN T-CELL LYMPHOTROPIC VIRUS TYPE I: POSSIBLE ETIOLOGICAL AGENT OF TROPICAL SPASTIC PARAPARESIS. P. Rodgers-Johnson, C. Mora, V. Zaninovic, O. St.C. Morgan, R. Biojó, M. Ceroni, D.M. Asher, R.M. Garruto, P. Sarin, C.J. Gibbs, Jr. and D.C. Gajdusek, National Institutes of Health, Bethesda, Maryland, USA, Hospital Universitario del Valle, Cali, Colombia, and University of the West Indies, Kingston, Jamaica.

IgG antibodies to HTLV-I have been estimated in sera and CSF of Jamaican and Colombian patients with tropical spastic paraparesis (TSP). By ELISA sera of 96% (27 of 28) Colombian and 77% (27 of 35) Jamaican TSP patients were positive at a dilution of ≥1:20. CSF of 92% (22 of 24) Colombian and 67% (21 of 32) Jamaican patients were similarly positive. These findings were confirmed by western blots which showed that IgG antibodies were most often directed against the p24 and p19 gag-encoded proteins of HTLV-I. Oligoclonal banding exclusively in CSF was detected by agarose isoelectric focusing and IgG immunoblot, suggesting an intrathecal inflammatory reaction. We also isolated HTLV-I from the blood of one TSP patient, and further attempts from both blood and CSF are in progress.

Tropical spastic paraparesis (TSP) is the commmonest neurological disorder seen in many tropical and subtropical countries. In the high-incidence foci of TSP in Jamaica and the Pacific littoral of Colombia, HTLV-I appears to be of etiological significance. The clinical features of patients from both these foci are predominantly those of spastic paraparesis, with Jamaican patients having more posterior column and spinothalamic tract involvement. Although HTLV-I and TSP are endemic to both Jamaica and Colombia, their distribution is islandwide in Jamaica but geographically localized in Colombia. Jamaica has a high incidence of adult T-cell leukemia, but similar findings have not yet been demonstrated for Colombia. Our data support the possibility that HTLV-I may be the cause of TSP in Colombia and Jamaica. Data from other laboratories for geographic foci in Martinique, Trinidad, Japan, the Seychelle Islands, and the Ivory Coast confirm this possibility.

R4.40

SEROEPIDEMIOLOGY OF HUMAN T-CELL LYMPHOTROPIC VIRUS TYPE I (HTLV-I) ANTIBODY IN POPULATIONS OF THE WESTERN PACIFIC. D.M. Asher, J. Goudsmit, R.M. Garruto, C. Mora, R. Yanagihara, C. Jenkins, K.L. Pomeroy, H. Askins, and D. C. Gajdusek. National Institutes of Health, Bethesda, Maryland, USA, and Institute for Medical Research, Goroka, Papua New Guinea .

IgG antibodies to HTLV-I were measured by enzyme immunoassay in more than 1800 sera collected between 1956 and 1986 in Papua New Guinea, West New Guinea (Irian Jaya), Bali, Indonesia, Solomon Islands, Vanuatu, Tuvalu (Ellice Islands) and the Mariana Islands (Guam), and among Australian aborigines from the Cape York Peninsula. Substantial differences in antibody prevalence were found between the various population groups. Serum of only one Balinese among 133 people from four villages had antibodies. Among the Asmat population from the Casuarine Coast of West New Guinea, 16% of sera were positive, while only 2% and 1% of sera, respectively, from the Bilogai and Mulia regions in the Central Highlands had antibodies. The Kapau Anga from the Gulf Province of Papua New Guinea had 6% while the Baruya Anga from the Eastern Highlands Province had no positive sera. 16% of sera collected from the Sulka people on the Gazelle Peninsula of New Britain and 15% from New Hanover were positive. Among three populations in the southern Solomon Islands (all Polynesian outliers), the percentage of seropositivity varied from 1% and 2% on Tikopia and Anuta, respectively, to 19% on Rennell Island. Among two populations in Vanuatu, the prevalence of seropositivity ranged between 9% and 16%, respectively, for Ureparapara and Toga Islands. Only 2% of sera from Micronesians on Ellice Islands were positive, and only 1% on Guam. No antibodies to HTLV-I were found in 151 sera from Cape York Peninsula aborigines. Relationships between the presence of antibodies to HTLV-I and various neurological diseases in these populations are being investigated.

R4.41

TROPICAL SPASTIC PARAPARESIS (TSP) CAUSED BY HUMAN T-CELL LYMPHOTROPHIC VIRUS TYPE-I (HTLV-I): INTRATHECAL SYNTHESIS OF IgG ANTIBODIES AGAINST HTLV-I IN TSP PATIENTS. M. Ceroni, P. Piccardo, C. Mora, P. Rodgers-Johnson, R.M. Garruto, C.J. Gibbs, Jr. and D.C. Gajdusek, National Institutes of Health, Bethesda, Maryland, USA.

Isoelectric focusing analysis of serum and cerebrospinal fluid (CSF) from Jamaican and Colombian patients with tropical spastic paraparesis (TSP) demonstrated oligoclonal banding of total IgG with some bands common to serum and CSF and other present only in CSF. Using an immunoblot technique employing HTLV-I precoated nitrocellulose paper, we demonstrated the presence of several well defined anti-HTLV-I IgG bands in the sera and CSF of TSP patients. Some bands were common to serum and CSF others were exclusively present in CSF. These data show that in some TSP patients specific antibodies to HTLV-I are synthesized intrathecally. The detection of specific oligoclonal banding of IgG antibodies to HTLV-I virus in the sera and CSF of TSP patients is strikingly similar to the demonstration of the specific banding of IgG antibodies to measles virus in subacute sclerosing panencephalitis. These data provide further evidence in support of an etiological role of HTLV-I in TSP. There is a high prevalence and age related distribution of HTLV-I antibodies in regions where high incidence foci of TSP and adult T-cell leukemia/lymphoma occur. We have detected by ELISA and Western blot of HTLV-I antibodies in 77-96% of sera and 67-92% of CSF of Jamaican and Colombian TSP patients, respectively, compared to an average of < 10% in the sera of non-neurological controls from endemic TSP areas. We have also isolated HTLV-I from the blood of a Jamaican TSP patient. Finally, spinal cord pathology in two Jamaican TSP patients showing typical features of an inflammatory demyelinating disease with perivascular cuffing of mononuclear cells (without any evidence of inclusion bodies) also suggests a primary viral infection.

R4.42

HUMAN T-CELL LYMPHOTROPIC VIRUS TYPE I ANTIBODIES IN HOUSEHOLDS WITH TROPICAL SPASTIC PARAPARESIS. R. Biojó, V. Zaninovic, C. Arango, R. Corral, M. Concha, I. Borrero, C. Mora, P. Rodgers-Johnson, R.M. Garruto, C.J. Gibbs, Jr., and D.C. Gajdusek. Hospital Universitario del Valle, Cali, Colombia and the National Institutes of Health, Bethesda, Maryland, USA.

IgG antibodies to HTLV-I were determined by enzyme immunoassay and Western immunoblots in the sera of 11 households of patients with tropical spastic paraparesis (TSP) in the endemic region of Tumaco on the southern Pacific coast of Colombia. We studied 11 TSP index patients, 41 first degree relatives (40 offspring, 1 sibling), 7 spouses (4 spouses did not live in the household and were not bled), 6 second degree relatives (grandchildren) and 5 non-related individuals. Controls consisted of sera from 24 individuals from 13 non-TSP households and included 13 index controls matched for age, sex and geographic location to that of the index patients.

Among the 11 TSP households, 100% of the TSP index patients and 5 of 7 spouses tested (71%) were seropositive for antibody to HTLV-I. In one conjugal pair both spouses with TSP were seropositive; in 4 other households the spouse was also seropositive. Twenty percent (8/40) of offspring were positive and these were from 5 of the TSP households. These included a 13 year old boy with early clinical features of TSP whose parents have TSP and are seropositive and a 20 year old woman and her 34 year old brother, who have TSP and whose mother is also a seropositive TSP patient. Thus, we have two separate households each with 3 seropositive TSP patients simultaneously ill. Among the remaining 11 household members none had TSP, but 3 were seropositive including a son-in-law and a grandson of a seropositive TSP woman and the sister of a second TSP patient. Among the 13 non-TSP control households, only one individual, a 58 year old woman, had antibody to HTLV-I. These findings provide further evidence that HTLV-I may have an etiological role in TSP. Sexual or other intimate contact and intrauterine, neonatal or perinatal infections and as well as biting insects may be responsible for spread of the virus.

R4.43

HUMAN T-CELL LYMPHOTROPIC VIRUS TYPE I ANTIBODY DISTRIBUTION IN PATIENTS WITH CHRONIC NEUROLOGICAL DISORDERS OTHER THAN TROPICAL SPASTIC PARAPARESIS. C. Mora, P. Brown, R.M. Garruto, M. Ceroni, P. Rodgers-Johnson, O. StC. Morgan, R. Yanagihara, L. Goldfarb, D. Gurroy, C.J. Gibbs, Jr., and D.C. Gajdusek. National Institutes of Health, Bethesda, Maryland, USA, and University of the West Indies, Kingston, Jamaica

Human T-cell lymphotropic virus type I (HTLV-I), the etiologic agent of adult T-cell leukemia/lymphoma , is also suspected to cause a chronic myelopathy referred to as tropical spastic paraparesis (TSP) in several widely separated geographical areas, and has been reported to be associated with multiple sclerosis. We have investigated the prevalence of antibodies to HTLV-I in patients with chronic neurodegenerative disorders other than TSP.

IgG antibodies to HTLV-I were measured in the sera and/or CSF from 82 Guamanian patients with amyotrophic lateral sclerosis and parkinsonism-dementia together with 164 non-patient Guamanian controls (collected in 1963-1976), 10 patients with kuru from the Eastern Highlands of Papua New Guinea (collected in 1963-1976), 4 patients with Viluisk encephalomyelitis from the Iakut region of eastern Siberia (collected in 1979-1980), 45 Italian patients with multiple sclerosis (collected in 1982-1985), and 49 American and 7 Jamaican patients with polymyositis (collected in 1973 and 1986, respectively). As determined by enzyme immunoassay, Western immunoblot, and gel-agglutination, serological evidence of HTLV-I infection was found in one Guamanian patient and one Guamanian non-patient control, in two patients with multiple sclerosis, and in one American patient with polymyositis. By contrast, 6 of the 7 (86%) Jamaican patients with polymyositis had IgG antibodies against HTLV-I. These results argue against an association of HTLV-I infection with many chronic neurologic diseases, but continue to support the etiologic importance of HTLV-I in TSP. The high seropositivity rate among Jamaican patients with polymyositis demands further study.

R4.44

MOLECULAR CLONING OF MEASLES VIRUS GENOME, MAPPING OF THE L GENE REGION, AND IDENTIFICATION AND USE OF 5' END CLONES AS DEFINITIVE PROBES FOR MEASLES VIRUS NUCLEIC ACID SEQUENCES IN SSPE AND MULTIPLE SCLEROSIS. Joan Crowley, Benjamin Blumberg[a], Joseph Menonna, Barry Schanzer, Stuart Cook, and Peter Dowling. Neurovirology Laboratory, East Orange VA Medical Center, East Orange, NJ 07019, and [a]Departments of Neurosciences and Microbiology, UMDNJ-New Jersey Medical School, 100 Bergen Street, Newark, NJ 07103, USA.

The 3' and 5' ends of paramyxovirus genomes are critical in viral transcription and replication, and also for the replication of defective-interfering (DI) particles which are thought to mediate persistent infection. In particular, the late L gene region is uniquely important in the formation of "copyback" DI's which may contain only these sequences. Measles virus (MV) is an important human pathogen, causing both acute illness and persistent infection. A definitive test for MV in persistent infection of the human CNS therefore requires probes for MV 3' and 5' end sequences. We have constructed a recombinant DNA library encompassing the entire MV genome. Overlapping clones representing the N, P/C, M, F and H genes were identified by reference to previously characterized clones (Dowling et al., J.gen.Virol. 67:1987-1992, 1986) and their positions were defined by S1 nuclease mapping of intracistronic clones; the L gene region was characterized by site-to-site restriction mapping. Clones representing the 3' and 5' ends of the genome were identified by probing with "capped" genomic RNA, and their locations were confirmed by primer extension. High specific activity riboprobes made from 5' end clones gave strong signals by in situ hybridization over brain cells from patients with SSPE, a CNS disease known to be caused by persistent MV infection. In contrast, these probes were silent when tested on brain tissue from patients with multiple sclerosis (MS), a CNS disease of suspected MV etiology. Within the limits of our current understanding of paramyxovirus replication, this result formally excludes the possibility that MS may be caused by persistent MV infection of the brain.

R4.45

GLUCOCORTICOIDS RESTITUTE NEURONAL FUNCTIONS IMPAIRED BY PERSISTENT VIRAL INFECTION.
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Persistent infection of neuroblastoma cells with rabies virus hep flury strain causes specific damage to typical neuronal membrane located functions leaving vital cellular function unaffected. This is reflected by a reduced cellular capability to react to the binding of neurohormones and neurotransmitters to their specific membrane receptors with characteristic changes in the enzymatic activity of membrane receptor linked adenylatecyclase.

The infection of a human neuroblastoma cell line was, comparable to a well-studied murine hybrid cell system with similar neuronal properties, followed by a nearly complete loss in specific receptor function.

Preincubation of infected cells for at least 48 hours with glucocorticoids (hydrocortisone 10-7M) completely restituted the receptor dependent function. The beneficial effect of glucocorticoids on specific virus induced functional deficits in our system cannot be explained by a global activation of membrane receptor dependent functions.

R5.1

MOLECULAR HETEROGENEITY AMONG STRAINS OF BOVINE VIRAL DIARRHEA VIRUS (BVDV). R. Magar, P. Trépanier, and J. Lecomte. Centre de recherche en virologie, Institut Armand-Frappier, Laval, Québec, Canada H7N 4Z3.

Bovine Viral Diarrhea virus (BVDV) is currently classified as a member of the Togaviridae family. Although well known as an important pathogen of cattle, little information is available on the molecular nature of the virus in general and on the variability of the viral proteins among strains.

Cytopathic and non-cytopathic strains were analyzed by radio-immunoprecipitation and SDS-PAGE with BVDV specific antiserum. Electrophoretic patterns of immunoprecipitated infected cell lysates demonstrated extensive variation among polypeptides of different strains. Furthermore a major 80 K polypeptide was found to be present in all cytopathic but absent in non-cytopathic strains indicating that this protein could be implicated in the cyto-pathogenicity of BVDV in vitro. Electrophoretic profiles resulting from in situ proteolytic digestion of the 80 K polypeptide of several cytopathic strains showed a high degree of homology. It is anticipated that similar observations will be found in cytopathic and non-cytopathic field isolates.

R5.2

STUDIES OF PERIPHERAL BLOOD MONONUCLEAR LEUKOCYTES FROM CALVES WITH PERSISTENT BVDV VIRAEMIA USING CORRELATIVE IMMUNO-GOLD EM. H. Bielefeldt Ohmann[1], B.Block[1], W.C.Davis[2], I.Askaa[3] Dept.Vet.Virol. Immunol.,Royal Vet.Agric.Univ.,Copenhagen,DK[1],Dept.Vet.Microbiol. Wash. State Univ. Pullman, Wash., USA[2],DANDPATTS Glostrup,DK[3].

Infection of the bovine fetus with non-cytopathic Bovine Viral Diarrhoea virus (BVDV) during the first trimester of gestation frequently results in the establishment of a persistent infection and the birth of viraemic calves with apparent tolerance to the virus. Recently we demonstrated that the infection occurred in T-, B- and null cells as well as in blood monocytes. In order to further characterize the BVDV infected leukocyte subpopulations, attempts were made to identify the infected cells by immuno EM (IEM). Due to the extreme sensitivity of BVDV antigens to various EM-preparative steps, we resorted to correlative IEM. Enriched subpopulations of PBL from persistently infected calves were tested for the presence of BVDV by direct virus-isolation and cocultivation. Double-labelling immunocytochemistry was performed on cytospin preparations using monoclonal antibodies specific for bovine leukocyte antigens. Cells were labelled in the living state for the surface antigens by an immuno-gold procedure and subsequently prepared for EM. BVDV-like particles (40-60nm) were detected by EM in small cytoplasmic vesicles of the aforementioned cell types, identifiable by the gold marker. The occurrence correlated with the results of the direct virus isolation from cell lysates. Among other notable morphological alterations were occurrence of complex membrane formations and granules with an amorpheous content. Many monocytes and T-lymphocytes looked "activated". A tentative account of virus morphogenesis in leukocytes is presented.

R5.3

POSSIBLE DUAL VIRUS ETIOLOGY FOR LACTATE DEHYDROGENASE-ELEVATING VIRUS INDUCED POLIOMYELITIS. C.H. Contag, A.T. Haase, P.G.W. Plagemann.
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The induction of poliomyelitis by lactate dehydrogenase-elevating virus (LDV) is dependent upon several host factors including age, loss of immune competence and genetic predisposition. The only two genetic components which segregate with susceptibility to the neurological disease are multiple proviral copies of endogenous murine leukemia viruses (MuLV) and homozygosity of the n allele at the Fv-1 locus. RNA was extracted from tissues of 8 month old C58 mice at various times following an LDV infection and analyzed in northern blot hybridizations using an AKR MuLV proviral probe. In support of a role for endogenous MuLV in poliomyelitis we find a tissue-specific increased expression of MuLV mRNA in spinal cords of paralyzed mice. This increased expression correlates with the development of paralytic disease following an acute LDV infection. Since LDV RNA can also be detected in dying neurons of paralyzed mice, a dual virus etiology is proposed for this neurological disease. The mechanism of this interaction is being assessed.

R5.4

ANTIBODY MEDIATED PROTECTION FROM LACTATE DEHYDROGENASE-ELEVATING VIRUS (LDV) INDUCED PARALYTIC DISEASE. J.T. Harty, S.P.K. Chan, C.H. Contag, P.G.W. Plagemann.
Department of Microbiology, University of Minnesota, Minneapolis, Minnesota, U.S.A.

Primary infection of 6-10 month old C58 mice with LDV may result in the development of paralytic disease. Antibodies directed towards the envelope glycoprotein of LDV are capable of protecting mice from paralytic disease when present at the time of infection either as immune complexes or through passive transfer. Protection does not correlate with the virus neutralizing activity of the antiserum employed and is not associated with decreased viremia in protected versus control animals that develop paralysis. Preliminary results from in situ hybridization analysis using an LDV-specific, mixed cDNA probe demonstrated the presence of numerous infected non-neuronal cells in spinal cord tissues from both protected and paralyzed mice. Infection of anterior horn neurons, which has been shown to correlate in time and space with the development of paralysis, was only detected in control mice and not in protected animals. Thus, the mechanism of protection in this system may involve formation of immune complexes, which specifically prevent the infection of anterior horn neurons without interfering with infection of non-neuronal cells of the CNS or normally permissive host macrophages.

R5.5

IS IA THE RECEPTOR FOR LACTATE DEHYDROGENASE-ELEVATING VIRUS (LDV)? I.K. Buxton, J.T. Harty, P.G.W. Plagemann.
Department of Microbiology, University of Minnesota, Minneapolis, Minnesota, U.S.A.

IA, the major histocompatibility antigen of mice, has been reported to be the macrophage receptor for LDV. We found that resident peritoneal macrophages from Balb/c mice cultured for up to 10 days were permissive for LDV infection and replication although the cells were IA negative after the first 48 hours of incubation. During the first 12 hours in culture, less than 1% of the 5-10% IA+ macrophages were also infected with LDV determined by dual labeling. This procedure labeled the LDV RNA with ^{3}H uridine and labeled the IA antigen with mouse anti-IA^d mAb and either FITC or peroxidase conjugated anti-mouse IgG. Mice that were injected with concanavalin A or indomethacin showed an increase in the expression of IA antigens on peritoneal macrophages. However, a lower percentage of these macrophages became infected with LDV in vitro than in comparable cultures from unstimulated mice. Our results indicate that IA antigen does not represent a major receptor for infection by LDV.

R5.6

ELECTRON MICROSCOPY OF BOVINE VIRUS DIARRHOEA/MUCOSAL DISEASE VIRUS IN INFECTED CALF KIDNEY CELLS

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Electron microscopic examination of thin sections from calf kidney cell cultures infected with either one of 5 immunologically identified bovine virus diarrhoea/mucosal disease virus field isolates revealed the modification of the rough endoplasmic reticulum and the presence of intra- and extra-cellular virus particles. Intracytoplasmic particles were found mainly within distended reticular vesicles and were formed of a core and a not closely bound single unit membrane. The extracellular virus were spherical, uniformly sized particles and 40±3 nm (mean of 100 particles) in diameter. They contain a dense core 30 nm in diameter surrounded by a well adherent thin envelope. These BVD virus particles are morphologically similar to those virions observed with hog cholera virus in thin sections.

R5.7

Further characterization of different isolates of lactate dehydrogenase-elevating virus (LDV). M. Brinton and E. Gavin, The Wistar Institute, Philadelphia, U.S.A. Murine infections with the non-arbo togavirus, LDV, usually result in an asymptomatic persistent infection characterized by impaired clearance of certain plasma enzymes, especially lactate dehydrogenase and a lifelong viremia. Virus replicates in an undefined subpopulation of macrophages. After the first week of infection, virus is present in circulating, infectious immune complexes, but persistently infected animals do not develop immune complex-related disease. Because of technical difficulties inherent to LDV infections, LDV isolates have not been typed by classical serological methods. RNA oligonucleotide fingerprint analysis has proven to be a useful means for comparing LDV isolates. Four genotypes of LDV have thus far been distinguished. Fingerprint patterns of some isolates changed upon passage, whereas others were more stable. LDV isolates also differ in their biological phenotypes. LDV-C induces mouse strain-specific CNS disease at a higher incidence than other LDVs. In immunosuppressed C58 mice LDV-C replicates in ventral horn neurons as well as macrophages, as demonstrated by in situ hybridization and electron microscopy. In C57BR/cd mice, inflammatory lesions in CNS white matter are observed after LDV-C infection. An immunopathogenic mechanism may account for these lesions, since no infected CNS cells have yet been found.

R5.8

MONOCLONAL ANTIBODY TO EQUINE ARTERITIS VIRUS 30K STRUCTURAL PROTEIN. E.D. Chirnside, M.W. Lock, J.A. Mumford Equine Virology Unit, Animal Health Trust, Newmarket, England

Equine Arteritis Virus (EAV) is closely associated with medium and host cell components throughout in vitro culture and virus purification. Consequently virus preparations used to immunise mice prior to fusion experiments and for subsequent hybridoma screening are heavily contaminated with non-viral proteins. Specific anti-EAV antibody producing hybridomas were therefore selected by separate ELISA screens against sucrose gradient purified EAV (Bucyrus strain), and uninfected host cell extracts. Western blot analysis [by EAV-specific monoclonal antibody (MAb)] of EAV proteins on polyacrylamide gels confirms the existence of a 30k (p30) structural protein.

Further purification of sucrose gradient prepared EAV by affinity chromatography on a MAb-Sepharose 4B gel column shows enhanced purification of major viral proteins with molecular weights 13.5K, 16.2K, 42K and 59K and several minor proteins with m.w. ranging 10K-60K.

R5.9

TOPOGRAPHICAL AND FUNCTIONAL ANALYSES WITH MONOCLONAL ANTIBODIES AGAINST HOG CHOLERA VIRUS. G. Wensvoort Central Veterinary Institute, Lelystad, The Netherlands.

Thirteen monoclonal antibodies (MCA1-13) were produced against hog cholera virus (HCV). Competitive binding studies and antigen capture assays were employed to map the corresponding epitopes. An abstract model could be constructed in which the MCAS define at least eight different epitopes which are situated on four distinct antigenic domains (A,B,C,D) on one protein. The functional relevance of this model was assessed by the detection of variant strains, by neutralization studies with the MCAS alone and in combination, and by isolating variants which escaped neutralization.

The topographical arrangement of the epitopes, as constructed in the model, was fully corroborated by the functional assays. Strain variation was detected in the domains B, C and D. Domain A was consistently found in all (93) HCV strains tested. Neutralization was detected with MCAS defining domain A, B and C. Synergistic neutralization could be observed with MCAS from domain A and B, and A and C, but not within the domains. By neutralizing with MCA 7, an escape-variant could be isolated which was not recognized by all MCAS (2,3,7,9,10 and 11) from domain A. By immunoaffinity purification, SDS-PAGE and Western blotting it could be shown that all 13 MCAS detected a protein with a molecular weight of 46-50 kd.

R5.10

NUCLEOTIDE SEQUENCE AND EXPRESSION OF RUBELLA VIRUS 24S RNA ENCODING THE STRUCTURAL PROTEINS E1, E2 AND C. D. Clarke, T. Loo, I. Hui, P. Chong and S. Gillam. Department of Pathology, Faculty of Medicine, University of British Columbia, Vancouver, B.C., Canada.

Rubella virus consists of a single molecule of a 40S single stranded RNA of positive polarity. Virus-infected cells contain, in addition to the genomic 40S RNA, a subgenomic 24S RNA that is derived from the 3' one-third of the genomic 40S RNA. The subgenomic 24S RNA is translated into a precursor to the structural proteins which is proteolytically processed to give the structural proteins C, E2 and E1.

The complete nucleotide sequence of the 24S RNA of wild-type M$_{33}$ strain rubella virus has been determined. This RNA is 3,383 nucleotides in length excluding the 3'-terminal poly(A) tract. After the three multiple in-phase termination codons clustered in the 5'-terminus of this RNA, there are 81 nucleotides of nontranslated nucleic acid followed by a reading frame of 3,180 nucleotides that encodes the 110 kD precursor of the structural proteins. Sites of post-translational cleavage to produce E2 and E1 have been located. RNAs synthesized by transcription in vitro are effective messengers in the rabbit reticulocyte cell-free translation system. Post-translational processing of the structural proteins was observed in the cell-free system supplemented with microsomes from dog pancreas. Eukaryotic expression vectors have been used to express each individual structural protein.

R5.11

POLYPEPTIDES OF BOVINE VIRAL DIARRHEA VIRUS AND THEIR VARIATION IN DIFFERENT STRAINS. R. K. Akkina and K. P. Raisch, Department of Microbiology, Colorado State University, Fort Collins, Colorado, 80523, U.S.A.

Bovine Viral Diarrhea (BVD) is an important disease of cattle worldwide. The causative agent is a RNA virus that belongs to pestivirus genus of Togaviridae family. We have identified at least ten viral specified polypeptides in infected cells. The molecular weights of these range from 14 kd to 250 kd. Using peptide mapping procedure we obtained evidence for post-translational proteolytic processing by this virus. Differences were observed between the cytopathic and noncytopathic strains in post-translational proteolytic processing. We also identified the viral glycoprotein responsible for inducing neutralizing antibody in the infected animal. Immunoprecipitation of nonglycosylated forms of viral glycoprotein obtained by tunicamycin inhibition of glycosylation indicated that the neutralizing epitope resides on the primary polypeptide backbone. Our results also indicate strain specific differences in the viral polypeptides among various strains suggesting genetic diversity. Differences in glycosylation of glycoproteins were observed among strains. The importance of strain variation in relation to varied pathogenicity and severity of disease will be discussed.

R5.12

MOLECULAR ORGANIZATION OF THE BOVINE VIRAL DIARRHEA VIRUS GENOME. Marc S. Collett, Ruby Larson, and Susan Belzer. Molecular Genetics, Inc., Minnetonka, MN 55343, USA.

Bovine viral diarrhea virus (BVDV) is implicated as a major contributing factor in the bovine respiratory disease complex, as well as being the causative agent of virus-induced diarrhea-mucosal disease in cattle. We have undertaken studies aimed at understanding the basic molecular characteristics of BVDV. The genome of BVDV (isolate NADL) is a highly structured, positive-strand RNA (lacking 3' terminal poly(A)) of approximately 12.5 kb in length. Having molecularly cloned this RNA, we are proceeding with DNA sequencing the cDNA clones and elucidating the coding capacity and expression strategy of this genome. In this latter respect, select regions along the genome have been manipulated so as to express BVDV sequences as polypeptides in E. coli. Antisera generated against these bacterially-produced BVDV analogue polypeptides were used to identify the authentic BVDV proteins to which they were directed. The resulting data has allowed for the identification and genomic positioning of most (all) of the virus-encoded gene products and suggests certain strategies for their expression. Furthermore, the genetic organization of the BVDV genome more closely resembles that of members of Flaviviridae, indicating that BVDV should no longer be considered as a togavirus.

R5.13

ARE THE INTRACELLULAR EQUINE ARTERITIS VIRUS (EAV) SPECIFIC RNAs GENERATED BY SPLICING? W.J.M. Spaan, E.J. Snijder, M.F. van Berlo and M.C. Horzinek. Institute of Virology, Veterinary Faculty, State University Utrecht, Yalelaan 1, 3508 TD Utrecht, The Netherlands.

Equine arteritis virus (EAV), although structurally similar, differs completely from other togaviruses in its mode of replication. In addition to the genome (RNA1; 4.3 x 10 mol.wt) 5 subgenomic RNA's are present in infected cells with the following molecular weights: 1.3 x 10^6 (RNA2), 0.9 x 10^6 (RNA3), 0.7 x 10^6 (RNA4), 0.3 x 10^6 (RNA5) and 0.2 x 10^6 (RNA6). The RNAs have a messenger activity but so far only a few viral proteins have been mapped to the RNAs. The genomic and subgenomic RNA's form a nested set sharing 3' sequences. UV transcription mapping experiments demonstrated that the EAV RNAs do arise from a common precursor. Hence, splicing seems to be a possible mechanism. Sequence analysis of the 3' end of the genome and the intracellular EAV RNAs are in progress to establish more precisely the homologies between the intracellular RNAs and the points of divergence. Since EAV is an RNA virus, replication in the cytoplasm, such a splicing proces should be an unorthodox one.

R5.14

FURTHER CHARACTERIZATION OF THE CELL FUSION AGENT (CFA) OF CULTURED MOSQUITO CELLS. R.K. Durbin, V. Stollar. Department of Molecular Genetics and Microbiology, UMDNJ-R.W. Johnson Medical School, Piscataway, NJ, USA 08854

Previous reports from this laboratory described an enveloped, positive strand RNA virus (CFA) originally isolated from cultures of Aedes aegypti cells. CFA caused massive fusion of Aedes albopictus cells. We now have plaque purified isolates of this agent, and have characterized it more fully. The molecular masses of the structural proteins, determined by SDS-PAGE, were: 52,000 and 16,000 for two major proteins and 49,000 and 20,000 for two minor proteins. The two smaller proteins are similar in size to those of the LDH virus. At least three stable virus specific nonstructural proteins (M_r = 77000, 63000, 26000) were found in infected cells. CFA clones varied greatly in their effects on infected cells. One isolate (CFA-C3) produced massive fusion and nearly complete cell killing within 24 hr. This isolate also regularly produced yields approximately 100 times the yield of the uncloned agent. Another clone resulted in an almost inapparent infection, though the virus yields were within tenfold of those produced by CFA-C3. CFA is strictly temperature sensitive in Ae. albopictus cells; no isolates, selected at 28°, were able to replicate at 34.5°. It is also host restricted; no isolates replicated in BHK cells.

R5.15

SEQUENCE OF THE 3' TERMINAL 3100 NUCLEOTIDES OF RUBELLA VIRUS RNA. T.K. Frey and L.D. Marr, Department of Biology, Georgia State University, Atlanta, Georgia, U.S.A.
The 3' terminal 5000 nucleotides of the rubella genomic RNA have been cloned and the 3' terminal 3100 nucleotides has been sequenced. This region contains the coding sequences for the virion proteins which are translated from a single open reading frame in the order NH_2-C-E2-E1-COOH. An open reading frame is present which stretches from the 5' end of the sequence to a termination codon 57 nucleotides from the 3' end. Within the open reading frame are the amino-termini of glycoproteins E2 and E1 as determined by Kalkkinen et al. (J. Gen. Virol. 65, 1549, 1984). The sequence of E1 has previously been reported (Frey, et al., Virology, 154, 228, 1986). The coding region for E2 predicts a polypeptide of 255 amino acids in length which contains four potential N-linked glycosylation sites and a potential hydrophobic membrane anchor near the carboxyterminus. Upstream from E2 is a stretch of 23 hydrophobic amino acids which has the features of a signal sequence. The content of this signal sequence is similar to the carboxyterminal sequence of the C protein as determined by Kalkkinen et al.(1984), indicating that the C protein is cleaved from the polyprotein precursor by signalase. From the 5' end of the open reading frame to the aminoterminus of E2 is a deduced amino acid sequence of 240 amino acids which is putatively the carboxyterminal 75% of the C protein.

R5.16

ANALYSIS OF MONOCLONAL ANTIBODIES TO BOVINE VIRAL DIARRHEA (BVD) VIRUS. E. J. Dubovi, R. O. Donis, W. Corapi. Diagnostic Laboratory, New York State College of Veterinary Medicine, Cornell University, Ithaca, NY, USA.

A panel of murine monoclonal antibodies(Mabs) specific for the two glycoproteins of the Singer strain of BVD virus was produced and analyzed by serum neutralization, radioimmunoprecipitation, and immunoblotting. Their protein specificity and their neutralization index divided the monoclonals into at least three classes: Class 1 Mabs were specific for the 56-58K glycoprotein and neutralized the virus. Class 2 Mabs were also specific for the 56-58K glycoprotein, but could not or poorly neutralized the virus. Class 3 Mabs recognized the 45K glycoprotein and could not neutralize the virus. These results clearly demonstrate that the 56-58K glycoprotein contains key antigenic determinants of the virus.

The panel of Mabs was used to probe the antigenic diversity of BVD viruses isolated from animals undergoing acute BVD infections, animals persistently infected with BVD virus, and animals that died of mucosal disease. The results of these studies indicate that the antigenic diversity seen within the biotypes (cytopathic and non-cytopathic) is just as great as that seen between the biotypes. The antigenic characteristic of viruses involved in mucosal disease will be discussed.

R5.17

MOLECULAR COMPARISON OF RV STRAINS. J.K. Chantler, K. Lund, C. Berkowitz, G. Tai, Department of Medical Microbiology, Faculty of Medicine, University of British Columbia, Vancouver, Canada.

All RV strains cross-react serologically but variations are known to occur in the type of plaque formation and in the degree of cytopathogenicity in culture. In vivo, different vaccine strains have been reported to vary in their ability to elicit a good immune response and in their association with side effects including joint symptoms.

A detailed comparison of the molecular difference associated with these biological variations has been carried out using the vaccine strains HPV 77/DE5 and RA 27, several wt+ strains (M33, Thomas, Therien, and 1B2) and a number of RV isolates from patients with chronic joint symptoms. The profile of viral polypeptides induced by each virus type in VERO cells and RAJI cells has been characterised on 1D SDS-gels, Western blots and on 2D gels. Reactivity with both polyclonal anti-RV sera and monoclonal antibody to E1 has also been determined. These experiments have shown that variations exist in both the E1 and E2 glycoproteins of the different strains. Tissue tropisms of the wt+ and vaccine strains have also been examined in a human macrophage cell-line, human lymphoblastoid cell-lines and human chondrocytes. A proclivity of all strains for human lymphoreticular cells has been identified. However, variation was found in the permissiveness of chondrocytes to the RA 27 strain, as opposed to wt+ and HPV77/DE5.

R5.18

SELECTION OF MONOCLONAL ANTIBODIES STRICTLY DIRECTED AGAINST BOVINE VIRAL DIARRHOEA VIRUS.
A.F. Lambert, M. Sneyers, M. Delchambre, E. Thiry, J. Dubuisson, J.J. Pin, G. Chappuis, P.-P. Pastoret. Service de Virologie - Immunologie et Pathologie des Maladies virales, Faculté de Médecine vétérinaire de l'Université de Liège, Bruxelles, Belgique. Rhône-Mérieux, Laboratoire IFFA, Lyon, France.

Monoclonal antibodies (Mabs) directed against Pestiviruses do often recognize cellular antigenic determinants. There is therefore a peculiar need for monoclonal antibodies strictly directed towards viral epitopes.
In order to segregate between monoclonal antibodies strictly directed against viral epitopes or not, we have used an indirect-immunofluorescence test to select our Mabs. Some of the Mabs which were obtained were further characterized and their neutralization capacity was tested.

R5.19

EXPERIMENTAL INFECTION WITH PESTIVIRUSES IN PREGNANT GOATS: T.Løken, National Veterinary Institute, and I. Bjerkaas, Norwegian College of Veterinary Medicine, Oslo, Norway.

62 pregnant goats were experimentally infected with pestivirus. Each goat was injected i.m. once with either a non-cytopathogenic ovine strain of border disease(BD) virus or a cytopathogenic strain of bovine virus diarrhoea(BVD) virus. The injections were made at 40, 60 or 100 days of pregnancy.

Neutralizing antibodies to BVD virus in titres ranging from 1:8 to 1:1024 were detected in all animals 5 weeks after inoculation. Out of the 21 goats injected at 40 days of gestation, three gave birth to dead or weak-born kids at full term. The remaining 18 animals suffered abortions or foetal deaths. 14 of the 23 goats injected on the 60th day delivered live but mostly weak-born kids at full term. There were three abortions and six foetal deaths in this group. In the final group of 18 goats there was one abortion. Of the 17deliveries at term there were three weak-born or dead kids.

The two virus strains did not seem to differ in ability to induce reproductive failure.

Histopathological lesions as seen in BD appeared in foetuses and kids, most frequently in the CNS. Virus was isolated from different organs of several offspring from nannies exposed to virus at all three stages of gestation. Most of the immunoenzymatically identified virus isolates were non-cytopathogenic. Precolostral neutralizing BVD antibodies were demonstrated in offspring from goats infected late in pregnancy.

R5.20

Characterization of Hog Cholera Virus (HCV) RNA. R.J.M. Moormann, Central Veterinary Institute, Lelystad, the Netherlands.

Upon analysis on formaldehyde-agarose gels of the cytoplasmic RNA of SK-6 cells infected with HCV strain Brescia in the presence of antinomycin D, a single 12.0 kb DNaseI resistent RNase A sensitive band could be detected which was not present in mock-infected cells. No obvious subgenomic RNA, which is found with the other Togaviruses, could be identified.

The 12.0 kb RNA sediments at 38S in an isokinetic formamide-sucrose gradient and has, if at all, a very short 3' poly-A tail as judged from poly-U sephadex chromatography.

Viral RNA which was isolated from the peak of infectivity of glycerol-tartrate gradient purified virus co-migrates with the 12.0 kb RNA from infected cells in neutral agarose-gels. Moreover, ^{32}P-labeled cDNA clones which were derived from the 12.0 kb RNA from infected cells, hybridize to this viral RNA species. These findings lead us to conclude that the HC viral RNA and its cellular counterpart are both present as a single RNA species of about 12.0 kilobases.

R5.21

RUBELLA SPECIFIC IgG SUBCLASS RESPONSES IN VARIOUS CATEGORIES OF RUBELLA INFECTION. P. Morgan-Capner and H.I.J. Thomas. Department of Virology, Preston Infirmary, Preston, PR1 6PS, U.K.

An ELISA technique has been devised to detect rubella-specific IgG subclasses. An attempt to surmount the problem of expression of results has been made.

Initial results suggest that in unvaccinated males with remote rubella the specific IgG is mainly of the IgG1 with trace amounts of IgG3 subclass. The response to vaccine is almost exclusively confined to the IgG1 subclass at about 2 months post vaccination. Two years after vaccination some cases show concentrations of IgG3 similar to those seen after remote natural infection.

However, in most sera from cases of recent rubella infection where rubella-specific IgM is detectable, there is an IgG1 and IgG3 response.

Examination of a small number of rubella reinfections has revealed an increase in IgG1, in all cases, and a slight rise in IgG3 in most cases.

At present, the results indicate the possible existence of a "trend" in IgG subclass response in different categories of infection, but no "absolute" pattern indicative of any one category.

The work is continuing and results from a larger number within each category, together with those from an examination of sera from cases of congenital rubella, will be presented.

R5.22

SOLUBILIZATION AND CHARACTERIZATION OF THE ERYTHROCYTE RECEPTOR FOR RUBELLA VIRUS. *P. Mastromarino, *L. Cioè, *F. Dianzani, *M. Orsi. *Institute of Microbiology and *Institute of Virology, School of Medicine, University of Rome "La Sapienza", Rome, Italy.

Goose erythrocyte membranes were isolated and tested for their ability to compete with red cell receptors for rubella virus attachment and fusion at acidic pH. Crude membranes, solubilized with Triton X-100, Tween 80 and octyl-β-D-glucopyranoside, showed a dose-dependent inhibitory effect on viral binding and hemolysis. The biochemical nature of the active molecules was investigated by enzyme digestion and by separation of purified components. Total lipids, extracted from membranes, were found to inhibit rubella virus attachment at a concentration of 31 μg/ml, whereas 1.5 mg/ml of membrane extracted proteins were devoid of any inhibitory effect. Among the various purified lipids tested only phosphatidylserine and sulfatides were able to inhibit viral hemagglutination and hemolysis. The involvment of membrane lipids in erythrocyte receptors for rubella virus was further evaluated by enzymatic digestion of whole erythrocytes.

R5.23

NUCLEOTIDE SEQUENCE OF THE GENES ENCODING THE RUBELLA VIRUS STRUCTURAL PROTEINS. G. Vidgren, K. Takkinen, N. Kalkkinen, A. Kallio, L. Kääriäinen, K.F. Pettersson[1]. Recombinant DNA Laboratory, University of Helsinki, Finland, and [1]Ludwig Institute for Cancer Research, Stockholm Branch, Sweden.

Our previous work has shown that rubella virus (RV) contains three structural proteins, E1 and E2 (membrane glycoproteins) and C (nucleocapsid protein). These proteins are made as a precursor and are encoded by a 24S (about 3500 bases long) subgenomic mRNA in the order NH_2-C-E2-E1-COOH.

Using RV 40S genomic RNA as a template, cDNA corresponding to the 24S mRNA region (3' one third of the 40S RNA) was synthesized and cloned in E.coli using pBR322 as a vector. Four partially overlapping clones covering the 24S mRNA region were sequenced and the genes for C, E2 and E1 were localized with the aid of the known aminoterminal amino acid sequences of E2 and E1 (Kalkkinen et al., 1984).

An open reading frame extending from the N-terminus of E2 to the C-terminus of E1 was identified. Mature E1 comprises 481 amino acids. This leaves a maximum of 283 residues for E2. The distribution of hydrophobic domains, potential glycosylation sites, possible proteolytic cleavage sites, as well as the relationship to other Togaviruses will be presented. Sequence data on the capsid protein will also be presented and discussed.

R5.24

BORDER DISEASE: THE DIFFERENT EFFECTS OF NON-CYTOPATHIC AND CYTOPATHIC VIRUS IN PREGNANT SHEEP. P.F. Nettleton, J.S. Gilmour, R.M. Barlow, A.C. Gardiner. Moredun Research Institute, Edinburgh, EH17 7JH, Scotland.

A pool of brains from lambs born to ewes experimentally infected with border disease virus (BDV) from a field outbreak was shown to contain both non-cytopathic (NCP) and cytopathic (CP) variants of the virus. The effects of cloned stocks of NCP and CP BDV were investigated by infecting ewes at 52 days gestation.

Infection with NCP virus resulted in barrenness, abortion, stillbirths and the birth of clinical 'hairy-shaker' lambs; NCP virus was recovered from all lambs and fetuses examined, but no precolostral specific antibodies were detected. In contrast, ewes infected with CP virus all went to term and produced apparently normal lambs; no BDV was recovered from these lambs but several had demonstrable specific antibody at birth. Histopathological lesions consistent with BDV infection were found in the CNS of lambs or fetuses from both groups but a major difference between groups was observed and this correlated with virological, serological and clinical differences. It was a surprising finding that major CNS lesions were present in clinically normal lambs.

These results further confirm that the two variants of BDV possess different biological properties in vivo as well as in vitro.

R6.1

STUDIES ON THE RELATIONSHIP BETWEEN CAULIFLOWER MOSAIC VIRUS MULTIPLICATION AND HOST FOLIAR DEVELOPMENT. A.J. Maule, C.L. Harker, I. Wilson. Department of Virus Research, John Innes Institute, Colney Lane, Norwich NR4 7UH, U.K.

The relationship between the multiplication of cauliflower mosaic virus (CaMV) and leaf growth/expansion was examined in the host plant, turnip. CaMV-infected turnip plants were dissected and all the leaves separately assayed for viral DNA, by hybridisation, and for the protein products of CaMV genes I-VI by Western blotting. Preliminary analysis indicated that the gene products were most successfully detected in either preparations of virus inclusion bodies (II, III, IV and VI), or in preparations of virus replication complexes (I, IV and V).

CaMV DNA was detected in the smallest leaves examined and increased in concentration thoughout the period of leaf expansion, and, probably, after cell division had been completed. CaMV gene products, detected in crude extracts of leaf tissue or in the two subcellular preparations, accumulated in parallel with viral DNA. However, for some virus-specific proteins, and in particular, coat protein (gene IV), the spectrum of products detected varied with the age of the leaf. For coat protein, processing of the primary translation product to the native protein was observed. These findings will be discussed in relation to the development of disease symptoms in CaMV-infected turnips.

R6.2

STUDIES ON THE REPLICATION OF CYMBIDIUM RINGSPOT VIRUS AND ITS SATELLITE J. Burgyán+, M. Russo Research Institute for Plant Protection, Budapest Hungary+ and Dipartimento di Patologia Vegetale, Universita di Bari, Italy.

A complete clone of cymbidium ringspot virus /CyRSV/ genome was prepared by primer extension of a clone previously constructed by oligo/dt/ priming of virus RNA polyadenylated in vitro. Subclones of regions corresponding to different parts of virus genome were also prepared by excising fragments and inserting them in plasmids. RNA preparations were obtained from infected plants and virions, and analysed by "Northern" blot hybridization. In addition to two subgenomic RNA species known to occur in plants inoculated with CyRSV deprived of its satellite, an additional smaller RNA species was identified in plants infected with CyRSV plus satellite. Such RNA was found to originate both from the 3' region of virus genome and a portion close to the 5' terminus. This RNA species is most likely involved in the modulation of virus expression. The two larger subgenomic RNAs occur both as single-stranded and as double-stranded form, the small one only as single-stranded. Specific translation products were identified by different methods. Satellite RNA has only linear molecules, both single- and double-stranded. No circular forms were detected after bidimensional gel-electrophoresis.

R6.3

EXPRESSION OF ALFALFA MOSAIC VIRUS (AMV) RNA4 IN TRANSGENIC PLANTS CONFERS VIRUS RESISTANCE. L.S. Loesch-Fries, D. Merlo, T. Zinnen, L. Burhop, K. Hill, K. Krahn, N. Jarvis, S. Nelson, E. Halk. Agrigenetics Advanced Science Company, 5649 East Buckeye Road, Madison, WI USA.

AMV is a multicomponent virus whose genome is comprised of 3 RNAs. A subgenomic RNA4, derived from RNA3, encodes the coat protein, which is essential for encapsidation and for initiation of virus replication. Transgenic Nicotiana tabacum plants that expressed AMV coat protein under control of the cauliflower mosaic virus 19S promoter were produced by Agrobacterium-mediated transfer of the gene from a binary vector. Tests with protoplasts from the transgenic plants showed that the coat protein was biologically active in initiating replication of AMV RNA1, 2, and 3 in the absence of RNA4 or exogenous coat protein. Plants that expressed the highest levels of coat protein, however, were resistant to AMV infection in that they developed fewer primary infections following inoculation with two strains of AMV and developed systemic infection slower than did plants that did not express coat protein. Resistance was specifically against virions of the AMV strains. AMV RNA and the unrelated virus, tobacco mosaic virus, were as infectious on progeny that expressed coat protein as they were on progeny that did not.

R6.4

THE REPLICATIVE FORM OF THE ABUTILON MOSAIC VIRUS EXHIBITS A DIVIDED GENOME AND CHROMATIN LIKE STRUCTURE. A.M. Abouzid and H. Jeske. Institut f. Allg. Botanik, Arbeitsbereich Genetik, Univeristät Hamburg, FRG.

The abutilon mosaic virus (AbMV) belongs to the geminivirus group (Abouzid and Jeske,1986;Harrison 1985). In addition to the twin particles found in preparations of AbMV there are structures of highly compact globular configuration in the presence of 0.15M NaCl which can be separated by sedimentation through sucrose density gradients. These structures were converted under dilution into an open structure with about 12 characteristic beads per molecule. This corresponds to the distribution of 24 beads in SV40 minichromosome which has nearly double the length of the AbMV genome.

The DNA isolated from these structures was analysed by electron microscopy and different nuclease treatments. The results suggest that the chromatin like structures are unit-genome length duplices of covalently closed circular viral DNA. Furthermore the AbMV genome,like BGMV, TGMV, and CLV, is composed of two components of nearly identical size: 2700bp and 2750bp.

R6.5

CHARACTERIZATION OF TMV-INDUCIBLE TOBACCO PROTEINS AND GENES. J.F. Bol, J.A.L. van Kan, B.J.C. Cornelissen. Dept. of Biochemistry, State University of Leiden, The Netherlands.

cDNA clones were obtained corresponding to seven classes of mRNAs ("clusters A to G") that are induced by tobacco mosaic virus (TMV) infection of Samsun NN tobacco. These cDNA clones were used: (1) to characterize the corresponding proteins by hybrid-selected translation and immune-precipitation with specific antisera, (2) to analyse the number of genes for these proteins on genomic blots, and (3) to isolate genes for these proteins from a genomic library of Samsun NN tobacco. Cluster B, D, E, F and G were found to correspond to pathogenesis-related (PR) proteins that are known to accumulate in the intercellular space of TMV-infected tobacco. Cluster B was found to correspond to a family of 8 genes encoding acidic PR-1 proteins. Three of these genes were cloned and sequenced. Cluster G was found to correspond also to a family of approximately 8 genes encoding basic proteins with a high degree of homology to the PR-1 proteins. Several cluster C genes were isolated from the genomic library and two of these were sequenced. A characterization of the proteins and genes corresponding to clusters D, E and F will be presented.

R6.6

IN SITU DETECTION OF THE TRANSLOCATION OF VIRUS IN PLANTS. B.J.M. Verduin, J.W.M. van Lent. Department of Virology, Agricultural University, Wageningen, the Netherlands.

Immunogold labelling of sections for light microscopy has been applied to determine the ultrastructural localization of cowpea chlorotic mottle virus coat protein antigen in cowpea plants (Vigna unguiculata cv. California Blackeye) as function of the infection periode. Cowpea plants were systemically infected with the differential temperature inoculation technique of Dawson and Schlegel (Intervirology 7 (1976) 284-291). Time zero corresponded with the shift of the trifoliate leaf to 27°C. Plant tissue was fixed with glutaraldehyde/paraformaldehyde and embedded in LR White. A silver enhancement was applied to the gold label to improve detection.

In the petiolules of systemically infected secondary leaflets, viral antigen was detected at t=0 in three phloem cells per cross-section. This number increased with time and at t=12 h antigen was also detected in endodermis cells and the adjoining cells of the cortex. At 24 h the number of labelled cortex cells had increased and some xylem vessels were labelled. Similar observations were made in cross-sections of the major veins of the leaflets. Furthermore patches of labelled mesophyll cells were found around vascular tissue as early as 12 h. These results led us to the conclusion that virus was transported through the phloem.

R6.7

INDIRECT EVIDENCE FOR REPLICATION OF COWPEA MOSAIC VIRUS RNA IN IMMUNE COWPEAS. P. Sterk and C.P. de Jager. Department of Virology, Agricultural University, Wageningen, the Netherlands.

In a cowpea line immune to cowpea mosaic virus (CPMV) this virus inhibited infection by the virulent cowpea severe mosaic virus (CPSMV), a member of the same virus group. The inhibition was complete if CPMV was added to the inoculum in 50-fold excess of CPSMV. UV-inactivated but intact CPMV-particles and CPMV-particles devoid of RNA (top component) did not inhibit CPSMV. Isolated CPMV-RNA inhibited infection by CPSMV and by isolated CPSMV-RNA, but not after having been inactivated by UV-irradiation.

Middle component of CPMV, containing the smaller non-replicating genome fragment, did not interfere with CPSMV-infection. Bottom(B) component of CPMV, containing the genome segment capable of independent replication, did inhibit CPSMV to the same extent as unfractionated CPMV.

Members of other plant virus groups like cowpea aphid-borne mosaic virus and tobacco mosaic virus (a cowpea-infecting strain) were also inhibited by infectious CPMV, but not by glutaraldehyde-inactivated CPMV.

These findings strongly suggest that the interference is not due to competition for infectible sites but is associated with replication of at least B-component RNA of CPMV in subliminally infected cells of the 'immune' cowpeas.

R6.8

ANALYSIS OF BROME MOSAIC VIRUS RNA REPLICATION GENE FUNCTIONS USING TARGETED MUTATIONS IN EXPRESSIBLE CLONED cDNA. P. Kroner, D. Richards, P. Ahlquist. Biophysics Laboratory and Department of Plant Pathology, University of Wisconsin, Madison, WI 53706, USA.

Brome mosaic virus (BMV) contains an 8.2 kb genome that is divided among three messenger-sense RNAs. While all three viral RNAs are required for systemic infection in whole plants, RNAs 1 and 2 together are sufficient to direct viral RNA replication in barley protoplasts. The 1a (109 kd) and 2a (94 kd) proteins encoded by BMV RNAs 1 and 2 share extensive sequence homology with non-structural proteins of several plant virus groups and the animal alphaviruses, suggesting that the proteins which direct RNA replication in this broad set of viruses are structurally and functionally related. We are investigating the functions of the BMV 1a and 2a proteins by analyzing the effects of a large number of point substitutions and in-phase insertions introduced into cloned cDNA copies of BMV RNAs 1 and 2. The ability of in vitro transcripts from these mutant cDNAs to direct both (+) and (-) strand viral RNA synthesis in barley protoplasts at permissive and non-permissive temperatures was assayed by Northern blot analysis. We have identified several widely spaced temperature-sensitive mutations in addition to phenotypes which range from apparently wild type to apparently lethal. Experiments examining the precise step of the RNA replication pathway inhibited in each temperature sensitive mutant will be discussed.

R6.9

COWPEA CHLOROTIC MOTTLE VIRUS INFECTION FROM VIRAL cDNA CLONES AND GENE EXCHANGES WITH BROME MOSAIC VIRUS. R. Allison, M. Janda, P. Ahlquist. Biophysics Laboratory and Department of Plant Pathology, University of Wisconsin, Madison, WI, 53706 USA.

Cowpea chlorotic mottle virus (CCMV), like other members of the bromovirus group of plant viruses, has a genome divided among three RNAs of around 3.3, 3.0 and 2.2 kilobases. Oligonucleotide-primed full length cDNA copies of the CCMV genomic RNAs were cloned immediately adjacent to a T7 RNA polymerase promoter. After linearizing the resultant plasmids at a unique restriction site flanking the inserted cDNA, complete virion-sense copies of the CCMV RNAs can be transcribed in vitro. A mixture of such transcripts representing all three CCMV genomic RNAs is highly infectious to cowpea plants, a systemic host, and undergoes efficient RNA replication in barley protoplasts.

We have previously constructed similar infectious cDNA clones of brome mosaic virus (BMV), the closest known relative of CCMV. Inasmuch as their genomes are similar in size and organization and share considerable nucleotide sequence homology, it should now be possible to study both common and distinct properties of BMV and CCMV by exchanging portions of their genomes. Among other work, attempts to modify the distinct host ranges of these two viruses by exchanging selected genes and gene segments will be presented.

R6.10

SYNTHESIS OF INFECTIOUS VIRAL RNA BY IN VITRO TRANSCRIPTION OF FULL-LENGTH DNA COPIES OF COWPEA MOSAIC VIRUS RNA. R. Eggen, P. Vos, M. Jaegle, J. Wellink, J. Verver, R. Goldbach and A. van Kammen. Dept. of Molecular Biology, Wageningen, The Netherlands.

The genetic information of cowpea mosaic virus (CPMV) is divided amoung two positive-sense RNA molecules refered to as B-RNA(mol.wt, 2.04×10^6) and M-RNA(mol.wt, 1.22×10^6). Each RNA has a small protein, VPg, covalently linked to the 5'end and a poly (A) tail at the 3'end. Both RNAs are required for infectivity in plants but B-RNA can accomplish its own replication in cowpea protoplasts. A set of full-length DNA copies of B-RNA or M-RNA were cloned behind the T7 promotor in such a way that upon in vitro transcription B-RNA-or M-RNA-like transcripts were obtained with two extra nucleotides at the 5'end and 5 extra residues at the 3'end of the CPMV RNA sequence.

Upon inoculation of cowpea protoplasts the RNAs were found to be infectious although the specific infectivity was very low(1% compared to viral RNA). The number of infected protoplasts was 2-3 times higher when capped RNAs were used in the infectivity tests. Furthermore the transcripts of different cDNA clones differed in infectivity. By exchange of fragments of different clones we are examining this variation in infectivity. In addition we are trying to increase the specific infectivity of the in vitro transcripts by removal of the extra nucleotides at both ends.

R6.11

IN VITRO, INHIBITION OF BMV RNA SYNTHESIS BY COAT PROTEIN, ANTISENSE VIRAL RNA AND GENOMIC RNA FRAGMENTS. I.Furusawa, M.Horikoshi, and K.Mise. Department of Agriculture, Kyoto University, Kyoto, Japan.

In vitro, brome mosaic virus (BMV) RNAs could be synthesized by the replicase isolated from BMV-infected barley leaves. This is a useful system for study of the mechanism of viral RNA synthesis. In vitro, the synthesis of BMV RNA was interfered with the viral coat protein. Although the inhibition was due to the binding of viral RNA and coat protein, it was not necessary to form a complete virion. Similar phenomena of the inhibition of viral RNA synthesis in vitro were observed when the antisense of 5' terminus 1200 bases of genomic RNA 3 was added to the reaction mixture of in vitro RNA synthesis. On the other hand, the addition of antisense of 3' terminus 200 bases to the mixture inhibited RNA synthesis only when the fragments and genomic RNAs were treated with 65C for 5 min and followed by cooling gradually. The plus-strands of 3' terminus 200 bases which were proposed as a binding site of the replicase also inhibited the synthesis of genomic RNAs synthesis. From these results, it was expected that thesemacromolecule would play a role of inhibitors of viral RNA synthesis in vitro.

R6.12

CONSTRUCTION OF FULL-LENGTH cDNA CLONES OF TOBACCO RATTLE VIRUS TO OBTAIN BIOLOGICAL ACTIVE TRANSCRIPTS.

Gerco C. Angenent, Huub J.M. Linthorst, John F. Bol

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Tobacco Rattle Virus (TRV) is a positive stranded RNA plant virus with a bipartite genome. The longer genome fragment, RNA1, has a length of about 6300 nucleotides in all strains. The length of RNA2, however, varies from strain to strain and ranges from 1800 to 4500 bases.

We cloned full-length cDNA of RNA2 of a TRV strain (PLB), isolated from potato, with a length of 2195 nucleotides. The RNA2 molecule consists of the cistron for coatprotein and for a 16K protein cistron, which is also present at the 3'end of RNA1. The 3'terminal sequence of PLB-RNAs 1 and 2 is identical over a length of about 880 nucleotides.

In order to investigate viral functions, full-length transcripts were made from PLB2 cDNA clones subcloned behind the T7 promotor. The biological activity of these transcripts is being assayed in protoplasts.

R6.13
PROCESSING OF THE TOBACCO ETCH VIRUS POLYPROTEIN. J. C. Carrington and W. G. Dougherty. Dept. Microbiology, Oregon State University, Corvallis, OR, USA.
The proteins encoded by the genome of tobacco etch virus (TEV), a plant potyvirus, are derived by processing of a single polyprotein precursor of 346K. An expression system has been developed utilizing the TEV 5' non-coding region and initiator codon to direct efficient translation of synthetic transcripts encoding portions of the TEV polyprotein. By expression of a series of transcripts representing the 3'-terminal half of the TEV genome, we have identified the 49K nuclear inclusion protein as a viral protease responsible for several cleavages on the polyprotein, including its own autocatalytic excision. The proteolytic activity resides within the carboxyl half of the 49K protein, since deletion of the amino half has no detectable effect on processing. Cleavage occurs at Q-G or Q-S dipeptides which are preceded by a conserved set of five amino acid residues. To test the hypothesis that only a short sequence of amino acids mediates proteolysis, a set of synthetic proteins, which contained insertions of the seven amino acid residues constituting the 54K protein-coat protein cleavage site (E-N-L-Y-F-Q-G), were generated. These proteins were processed by the 49K protease, suggesting that recognition of the cleavage sites by the viral protease requires no more than seven residues on the substrate.

R6.14
COMPARATIVE SEQUENCE ANALYSIS OF CARNATION MOTTLE AND TURNIP CRINKLE VIRUSES: EVIDENCE FOR GROUP AFFINITY. T. J. Morris, J. C. Carrington, D. Buidema, B. I. Hillman and L. A. Heaton. Department of Plant Pathology, University of California, Berkeley, CA, USA.
The nucleotide sequence of more than 95% of the turnip crinkle virus (TCV) genome has been determined from cDNA clones and directly from the viral RNA. The sequence of the 3'-end has been confirmed by chemical RNA sequence analysis. Primer extension experiments were used to determine the 5'-end sequence, as well as to map the origins of two subgenomic RNAs of about 1.5 and 1.7 kb. Comparison of the TCV sequence with the previously determined sequence of carnation mottle virus has clearly demonstrated a close affinity of the two viruses. Each has three major open reading frames that encode proteins with evident homologies at the amino acid level. In contrast, comparison of the TCV sequence with the more recently determined partial sequence of the tomato bushy stunt virus (TBSV) genome convincingly demonstrates that the genomic organizations of TCV and TBSV are different. The implications of these results for the classification of TCV as a tombusvirus will be discussed.

R6.15
JUNCTIONS IN RECOMBINANT CAULIFLOWER MOSAIC VIRUSES AND SELECTIVE ALLELE LOSS. Valerie R. Vaden, Ulrich Melcher. Department of Biochemistry, Oklahoma State University, Stillwater OK 74078-0454 USA.
Junctions between parental DNAs in recombinant cauliflower mosaic viruses (CaMVs) were localized by restriction mapping and nucleotide sequencing. Recombinant CaMVs analyzed were obtained either from a population of a natural CaMV isolate (W from Cabb B-J1) or from inoculation of turnip plants with pairs of non-infectious mutant DNAs each derived from a different isolate. Identified junctions were preferentially, but not exclusively, localized to the vicinity of open reading frame III and gap 1. The junction at gap 1 likely resulted from reverse transcription during progeny DNA synthesis. The open reading frame III junctions were close to a locus at which selective loss of one of two coinoculated alleles occurred. The selective allele loss did not occur when the two coinoculated viruses were non-homologous in 5% of their residues. Non-homologies flanking both sides of the locus were sufficient to prevent selective allele loss. Allele loss occurred when non-homology was placed on only one side of the locus. The results suggest involvement of both reverse transcription and double-stranded DNA recombination in the exchange of sequence information leading to evolution of caulimoviruses. (Supported in part by the Oklahoma Agricultural Experiment Station and the Herman Frasch Foundation.)

R6.16
CHARACTERIZATION OF A MOSAIC TYPE OF DEFECTIVE INTERFERING RNA ASSOCIATED WITH TOMATO BUSHY STUNT VIRUS. Bradley I. Hillman and Thomas J. Morris. Dept. of Plant Pathology, University of California, Berkeley, 94720, USA.
The biological and physico-chemical properties of a low molecular weight RNA species capable of modulating symptoms associated with infection by the Prunus strain of tomato bushy stunt virus (TBSV; syn. petunia asteroid mosaic virus, PAMV) were investigated. This symptom-modulating RNA was shown by nucleotide sequence analysis to be comprised of a mosaic of sequences derived from various portions of the TBSV genome. Besides attenuating symptoms in virus-infected plants, this genome-derived RNA species led to a decrease in the amount of standard virus extractable from infected plants, relative to plants infected with only genomic RNA. It has therefore been called a defective interfering (DI) RNA, analogous to such RNAs associated with animal viruses. The 3' terminus of the TBSV genome was present on the symptom-modulating RNA, but sequences at the 5' termini of the two RNAs were different, the DI RNA lacking the 5'-terminal 10 nucleotides that were present on the viral genome. Other deletions were evident as well. In all, seven discreet stretches of genomic sequence, each varying in size and in amount of deleted intervening sequence, were represented on the DI RNA. This is the first mosaic type of DI RNA described in a plant virus system.

R6.17
STRUCTURE AND RELATEDNESS OF SATELLITE PANICUM MOSAIC VIRUS AND RELATED STRAINS. A. O. Jackson, C. Hasuta, B. G. Hunter, L. A. Heaton, D. Buidema, and D. Sopher. Department of Plant Pathology, University of California, Berkeley, CA 94720. USA.
The properties of the genomes of satellite panicum mosaic virus (SPMV) and a satellite associated with St. Augustine decline virus (SSAD) were investigated by gel electrophoresis. The results show that both SPMV and SSAD have a major component of about 800 NT with a smaller RNA component of 500 NT associated with some SSAD isolates. Sequence analysis of SPMV RNA reveals that the genome is 826 nucleotides (NT) long and the ability to label SPMV RNA with polynucleotide kinase only after phosphatase treatment suggests that the 5' terminus is phosphorylated. The first open reading frame (ORF), encountered after an 88 NT 5'-untranslated region, encodes a 17,000 molecular weight (MW) protein nearly identical in size and amino acid composition to the authentic capsid protein. An additional short ORF, located near the 3' end of the RNA, could encode a 5,500 MW polypeptide. The minus strand also contains two ORFs that could encode polypeptides of 6,500 and 11,000 MW.

R6.18
ANALYSIS OF THE SUBGENOMIC PROMOTER OF BROME MOSAIC VIRUS. *Loren E. Marsh, †Hans Van Bokhoven and *Timothy C. Hall, *Dept of Biology, Texas A. & M. University, College Station, Texas 77843 and †Department of Virology, Agricultural University, Wageningen, Netherlands.
Brome mosaic virus (BMV), a (+)strand RNA virus produces 4 mRNAs, 3 genomic and 1 subgenomic, RNA4. RNA4, the mRNA for the coat protein is generated from the 3' end of dicistronic genomic RNA3. Subgenomic (+)RNA can be synthesized in vitro on minus-strand RNA template by a replicase (RNA dependent RNA polymerase) extract from infected barley leaves. The subgenomic promoter for (+)RNA4 transcription has been delineated by the use of mutant templates. Nested ladders of deletion mutations were constructed in the cDNA transcriptional plasmids both upstream and downstream of the promoter. Analysis of these mutations has allowed the core sequence of the subgenomic promoter to be limited to about twenty bases upstream of the subgenomic initiation site. However, the complete promoter is considerably larger, about 61 bases and apparently contains at least 3 functional domains. Comparison of the promoter of BMV with the putative alphavirus promoters revealed several conserved blocks of homology. The promoter has been further analyzed by oligonucleotide site directed mutagenesis of these conserved bases.

R6.19

GENETIC ORGANIZATION OF RNA3 FROM BARLEY STRIPE MOSAIC VIRUS. B. G. Hunter, G. Gustafson, I. T. D. Petty, A. O. Jackson. Department of Plant Pathology, University of California, Berkeley, CA 94720, USA.

The genome of barley stripe mosaic virus (BSMV) consists of three (+) sense RNA molecules. The sizes of RNAα and β appear to be constant, but there is considerable strain variation in the length of RNA3. For example, the Type strain RNA3 sequence is 3164 nucleotides (NT) long, while the ND18 RNA3 is 2791 NT long. Both sequences contain a 5' non-coding region followed by two open reading frames (ORF) separated by a 42 base intercistronic region. While there is a high degree of homology between the two sequences, ORF1 from the Type strain contains a 366 nucleotide direct tandem repeat which is absent in the ND18 ORF1. As a result, the polypeptide encoded by the Type ORF1 (87kDa) is larger than the ND18 strain (71kDa). The predicted amino acid sequence of the ORF1 polypeptide contains homologies with putative RNA polymerases from other (+) sense RNA viruses, suggesting that this protein may also function in RNA replication. The ORF2 polypeptide is predicted to have a molecular weight of 17kDa and is nearly identical between the two strains. In order to raise antibodies to the putative proteins encoded by RNA3, sequences from ORF 1 and 2 have been expressed in E. coli as fusions with the lacZ gene.

R6.20

HORDEIVIRUSES: GENOME STRUCTURE AND EXPRESSION. J.G.Atabekov. Department of Virology,Faculty of Biology,Moscow State University,Moscow 119899,USSR

Barley stripe mosaic virus (BSMV)is the type member of hordeivirus group of plant viruses. Its genome consists of three separately encapsidated messenger-sense RNAs, RNA 1 (4.0kb),RNA 2(3.3kb) and RNA 3(2.8 to 3.2kb, depending on the virus strain).Formally,hordeiviruses belong to the tricornaviruses, however only BSMV RNA 1 resembles RNA 1 of tricornaviruses: it codes for a single replication related protein of Mr 12 oK. The BSMV coat protein gene is located in the 5'-terminal region of RNA 2 and is actively translated therefrom. RNA 3 is bicistronic: it codes for the BSMV putative replicase (74-86K) and 17K protein expressed by the subgenomic RNA in vivo.

One of the peculiarities of BSMV is a natural unstability of gene,coding for the 74-86K putative replicase:it can be elongated by duplication of some 350 nucleotides or shortened by deletion of 185 nucleotides.In the former case the resulting 86K protein is functionally competent,in the latter 60K protein seems to lose its activity.The 3'-terminal regions of BSMV RNAs contain a unique tandem of an internal poly(A)tract and terminal tRNA-like structure.

R6.21

INFECTION AND MULTIPLICATION OF RICE GALL DWARF VIRUS IN VECTOR CELL MONOLAYERS. T. Omura, I. Kimura, T. Tsuchizaki and Y. Saito. National Agriculture Research Center, Tsukuba Science City, Yatabe, Ibaraki 305, Japan

The system for the infectivity assay and synchronous multiplication of rice gall dwarf virus (RGDV)was established using the vector cell monolayers of the green rice leafhopper Nephotettix cincticeps. A linear relationship was observed between the number of infected cell foci stained with fluorescent antibody method and the virus concentration in various kinds of inocula; infected plants, viruliferous insects, infected monolayer cells and purified virus. The dilution end point for the detection of virus activity was $10^{-6.5}$ for the infected plants in the vector cell monolayer and the method was about 10^3 times more sensitive than enzyme -linked immunosorbent assay. The 100% infection of vector cell monolayer was achieved when samples more concentrated than the following dilutions were used as inocula; $10^{-3.5}$ for infected plant, $10^{-4.5}$ for viruliferous insects, $10^{-5.5}$ for infected monolayer cells and $A_{260}>10^{-5}$ for purified preparation. In the vector cell monolayers, the virus was first detected 10 hours after inoculation and then vigorously multiplied in 10^5 times in 30 hours afterwards. The high efficiency of virus infection and the synchronous virus multiplication demonstrate that the vector cell monolayer is an excellent experimental system for the study of the biological functions of RGDV.

R6.22

ANALYSIS OF REPLICATION IN TWO STRAINS OF CUCUMBER MOSAIC VIRUS. Marilyn J. Roossinck, Michael Shintaku, and Peter Palukaitis, Dept. Plant Pathology, Cornell Univ., Ithaca, NY, USA.

Two strains of cucumber mosaic virus (CMV) isolated in New York State exhibit phenotypic variation in the rate of systemic infection and appearance of symptoms in infected plants, although both strains eventually achieve comparable virus titers. We have utilized these two strains to study the gene(s) responsible for the control of replication of CMV. To distinguish replication rate from the rate of cell-to-cell movement we compared the accumulation of CMV RNA over time from tobacco protoplasts which had been electroporated with RNA from the fast (Fny) and slow (Sny) strains. Using pseudo-recombinants of purified RNAs 1, 2, and 3 from the two strains we will investigate the gene(s) responsible for rates of replication of Fny and Sny. By transfecting with RNAs 1 and 3, and 2 and 3 from strain Fny we are also mapping which RNA species are necessary and/or sufficient for RNA replication in protoplasts.

R6.23

COMPARISON OF THE GENOMES OF MELON NECROTIC SPOT AND FOUR SIMILAR VIRUSES. J.E. Pot[1], D.M. Rochon[2] and J.H. Tremaine[2]. Agricultural University, Wageningen, Netherlands[1], Agriculture Canada Research Station, Vancouver, B.C. Canada[2].

Melon necrotic spot (MNSV), carnation mottle (CarMV), tobacco necrosis (TNV), tomato bushy stunt (TBSV) and cucumber necrosis (CNV) are spherical plant viruses with particle diameters of ca. 30 nm and monopartite single stranded RNA genomes of 4.0 to 4.8 kilobases. Electrophoretic patterns of double stranded RNAs of MNSV, CarMV and TNV were found to be similar to each other but distinct from those of TBSV and CNV. This suggests that the genome organization of MNSV is similar to that of CarMV and TNV but differs from that of TBSV and CNV. To further analyze this, double stranded cDNA to MNSV was prepared and cloned. Restriction endonuclease analyses of two of these clones indicate that together they represent at least 95% of the genome. These two MNSV clones were used as probes in nucleic acid hybridization analyses. No hybridization was detected between these probes and either CarMV or TNV indicating a distant relationship, if any, between MNSV and both CarMV or TNV. The nucleotide sequence of the MNSV coat protein gene and another cistron of unknown function is currently being determined. The deduced amino acid sequences will be compared with the published amino acid sequences of CarMV proteins.

R6.24

CUCUMBER NECROSIS VIRUS IS A TOMBUSVIRUS. D.M. Rochon and J.H. Tremaine. Agriculture Canada, Research Station, Vancouver, B.C. V6T 1X2

Cucumber necrosis (CNV), tomato bushy stunt (TBSV), tobacco necrosis (TNV) and melon necrotic spot (MNSV) viruses are soil-borne isometric plant viruses with particle diameters of ca. 30 nm and monopartite, linear single stranded RNA genomes of ca. 4.5 kb. Natural spread of CNV, TNV and MNSV is facilitated by Olpidium zoospores. We assessed the relationship between CNV and the other viruses by comparison of ds RNA patterns and by nucleic acid hybridization analyses. The electrophoretic patterns of the ds RNAs of CNV and TBSV were identical and distinct from those of TNV and MNSV. The results of hybridization analyses using a full or nearly full length cDNA clone of CNV demonstrated extensive nucleotide sequence homology between CNV and TBSV but none between CNV and either TNV or MNSV. The reported lack of serological relationship between CNV and TBSV was confirmed in gel diffusion and in indirect ELISA tests with virus, swollen virus and virus protein antigens. Nucleic acid sequence homology between serologically unrelated plant viruses has not been reported previously. The nucleotide sequence of most of the coat protein cistron of CNV has been determined. The deduced amino acid sequence of CNV will be compared with the published amino acid sequence of TBSV.

R6.25

RAPID in vitro SCREENING SYSTEM FOR ANTI-PLANT VIRAL SUBSTANCES FROM PLANTS. Evamarie SANDER. Institut für Biologie II, Universität Tübingen,D-7400 Tübingen,Federal Republic Germany.

Substances impairing virus replication can be inherent in plants and/or virus induced.Detection,efficiency of purification and screening of antiviral potential is hampered by substance, time and labour consuming bioassays. To facilitate more rapid screening in vitro, Tobacco Mosaic Virus (TMV)and protoplasts(PP) from tobacco leaves and soybean cells in suspensionculture,and cells from a newly established tobacco cell line were compared as infection system. Actinomycin D served as reference virus-replication suppressor.The rate of TMV replication was comparable in the 3 systems as determined within 38 h of incubation by bioassay, with mono-and polyclonal antibodies in ELISA and RPH (reverse passive haemagglutination)a one-step virus detection giving results readable with unaided eyes within 15-90 min incubation within 4-37°C. RPH detected TMV with sensitivity equal to that of ELISA.The immuno-assays mirrored the TMV replication rate of the bioassay. In the in vitro PP system a proteinaceous antiviral factor from plants inhibited TMV replication at 1/800 of a dose effecting the same results when applied to leaves.Compared with leaf-PP,the PP and cells from suspension cultures are equally suitable with the advantage of availability in uniform quality throughout the year. The proposed in vitro system allows detection and screening of antiviral substances and assays of virus resistance in plants effected by breeding or gene-techniques.

R6.26

THE USE OF IN VITRO TRANSCRIPTION TO INVESTIGATE GENE FUNCTION IN COWPEA MOSAIC VIRUS.
G.P. Lomonossoff. Dept. of Virus Research, John Innes Institute, Norwich, U.K.

Full-length cDNA copies of the middle component (M) RNA of cowpea mosaic virus (CPMV) have been cloned in the transcription vectors pSPT18 and pPM1. In vitro transcription with either SP6, T7 or E.coli RNA polymerases results in the synthesis of RNA which co-migrates with M RNA isolated from virus particles. The transcripts also give the same polypeptides when translated in rabbit reticulocyte lysates as does authentic M RNA.

Clones harbouring the coat protein region of M RNA have been constructed in the transcription vectors pSPT18 and pSPT19. The clones have been so constructed that translation of the in vitro transcripts begins at the methionine residue known to form the N-terminus of the 60K precursor of the two viral coat proteins. These clones are currently being used to investigate the maturation of the coat proteins and the possible role of the 60K precursor in virus assembly, and the results of these investigations will be presented.

R6.27

ENCAPSIDATION OF NON-VIRION RNA SPECIES WITH TOBACCO MOSAIC VIRUS PROTEIN. C.D. Atreya, J. P. Gaddipati, A. Siegel. Department of Biological Sciences, Wayne State University, Detroit, MI U.S.A.

Mechanisms exist in host cells to ensure that viral components are assembled into functional virions. However, we have previously reported that TMV coat protein reacts with certain specific sequences (ei sites) on host RNA species to form psuedovirions. Among the RNA species which contain such sequences are 18S rRNA and many chloroplast DNA transcripts. We report here localization of ei sites on two of these RNA species, 18S RNA and RUBISCO large sub-unit mRNA. A 37 base sequence near the 5' end of 18S rRNA and a 164 base sequence in the middle of the RUBISCO large sub-unit transcript were found to contain ei sites. The nucleotide sequence of these regions were determined. In addition to the ei site on large sub-unit gene transcript, a strong block to encapsidation elongation was located on material transcribed from a region just 3' to the end of the RUBISCO gene. Our data indicate that ei sites occur in a number of non-virion RNA species, that position of an ei site is relatively unimportant for encapsidation and that even though the nature of the RNA being encapsidated during the elongation reaction is, in most cases, non-specific, some RNA sequences present a block to elongation.

R6.28

INDEPENDENT REPLICATION OF RED CLOVER NECROTIC MOSAIC VIRUS RNA-1 IN PROTOPLASTS. S.A. Lommel and L. Paje-Manalo. Department of Plant Pathology, Kansas State University, Manhattan, KS 66506.

Red clover necrotic mosaic virus (RCNMV), a member of the dianthovirus group, is composed of two nonhomologous RNAs of 4.1kb (RNA-1) and 1.4kb (RNA-2). RNAs are packaged into a single 30nm icosahedral virion by 180 identical capsid protein subunits of 39kd. RNA-2 is monocistronic and codes for a single 36kd polypeptide. RNA-2 is thought to be involved in allowing cell-to-cell movement of virus infection. RNA-1 cannot be detected when inoculated to plants. However, when RNA-1 is inoculated to whole plants, followed 48 hrs by reinoculation with RNA-2, a wild-type infection results. This indicates that RNA-1 replicates within the primary infected cells but does not move and infect adjoining cells. RNA-1 has been inoculated by electroporation to protoplasts of Nicotiana clevelandii and BY-2 tobacco suspension cell line. The replication of RNA-1 was observed in the protoplasts in the absence of RNA-2, and a chronic infection of RNA-1 in the BY-2 cells has been established. These experiments illustrate that RCNMV RNA-1 can replicate autonomously and indicate that RNA-2 is necessary for cell-to-cell movement.

R6.29

THE ROLES OF CIS AND TRANS ELEMENTS IN CASSAVA LATENT VIRUS INFECTION. B.A.M. Morris-Krsinich, M.T. Andersen and R.C. Gardner*. Plant Diseases Division, DSIR, Auckland, New Zealand; *Department of Cell Biology, University of Auckland, Auckland, New Zealand.

Cassava latent virus (CLV) is a representative of the whitefly transmitted geminiviruses which infect dicotyledonous plants. It has a bipartite genome comprising two similarly sized circles of single-stranded DNA (DNA 1, 2779 nucleotides and DNA 2, 2724 nucleotides) which share a region of sequence encompassing approximately 200 nucleotides.

CLV is being investigated as a model to develop a vector system for extrachromosomal replication and expression of chimeric genes in plants. The roles of *cis* and *trans* elements in CLV infection are being examined using the *Agrobacterium tumefaciens* Ti plasmid gene transfer system. Dimer forms (tandem direct repeats), monomer forms and deleted forms of each the CLV components have been introduced into *N. plumbaginifolia* cells by way of the leaf disc transformation procedure for plants to be regenerated. In addition the DNA forms have been introduced into whole plants by wounding and gall formation. Results of these experiments will be presented and discussed.

R6.30

IMMUNOCYTOCHEMICAL STUDY OF SOYBEAN MOSAIC VIRUS-INFECTED TISSUES. J. Vega. Virus Departament,Instituto Agronômico, Campinas, São Paulo, Brazil.

The most conspicuous cytopathic effect of soybean mosaic virus (SMV) infection is the formation of cytoplasmic inclusions (CI). The protein that forms the CI is encoded by the viral RNA genome and is unrelated serologically to the capsid protein.

Soybean leaf tissues were studied using the protein A-gold technique. The samples were processed as commonly for immunocytochemistry, but was used Araldite-Epon mixture for embedding and omitted the etching treatment of the sections.

The preservation of the cellular structures was quite good considering that osmication was not used. Heavy labelling of SMV particles was obtained, with light background immunostaining.

The cytoplasmic inclusions, despite some unevennes, appear consitently labelled. This labelling is not as intense as the labelling of SMV particles and seems to be located at the surface of the inclusion lamellae. Frequently appear CI, labelled or not, intruding cellular organelles previously identified as peroxisomes.

These results indicate a real association of virus coat protein with CI. It is possible, however, that this association is modulated during the replicative process of the virus.

R6.31

A MATURE INHIBITOR OF VIRAL PROTEIN PROCESSING. D.S. Shih, K.C. Scorsone, M.Bu, and C.T. Shih, Department of Biochemistry, Louisiana State University, and Department of Biological Sciences, Southern University, Baton Rouge, Louisiana. U.S.A.

In a rabbit reticulocyte lysate protein-synthesizing system, the two genomic RNAs of cowpea mosaic virus (CPMV), the M and B RNA, are translated into polyproteins which are cleaved to give mature viral proteins. We found that cowpea embryo and wheat embryo contain an activity which interferes with these specific cleavage reactions. The inhibitory activity in crude embryo extracts is most effective on the primary (initial) cleavage of the translation product of B-RNA. Higher concentrations of the inhibitor preparation are necessary to cause a similar level of inhibition of the secondary cleavages of B-RNA translation products and cleavages of the polypeptide encoded by M-RNA.

The protease inhibitor of the primary cleavage of the B-RNA encoded polypeptide has been partially purified from wheat embryo. The reactivity of this partially purified activity to several know proteases is currently being investigated. In addition, its specificy for the secondary cleavage reactions of CPMV polyproteins as well as the cleavage reactions of picornaviurs translation products is being tested.

R6.32

MECHANISM OF TURNIP YELLOW MOSAIC VIRUS (TYMV) COAT PROTEIN SUBGENOMIC mRNA SYNTHESIS IN VIVO*.

Radhia Gargouri, Rajiv L. Joshi, John Bol+, Anne-Lise Haenni. Institut Jacques Monod, 2 Place Jussieu, 75251 Paris, France and +State University of Leiden, P.O. Box 9505, 2300 RA Leiden The Netherlands.

TYMV possesses a single-stranded '+' sense RNA genome. The coat protein cistron is located at the 3' end and is expressed in vivo through a subgenomic mRNA. Two mechanisms can be envisaged for the synthesis of this subgenomic mRNA. 1. During transcription of the genomic RNA, premature termination could lead to a minus strand RNA of subgenomic length that could serve as template for subgenomic mRNA synthesis. 2. The subgenomic mRNA could be generated by internal initiation on a minus strand RNA of genomic length.

To distinguish between these possible mechanisms, total nucleic acids were isolated from TYMV-infected leaves and double-stranded RNAs (RF and RI) were purified. By gel electrophoresis of this material under native or denaturing conditions followed by Northern blot hybridization using a subgenomic mRNA probe, no minus strand RNA of subgenomic length could be detected. This strongly argues in favour of the second mechanism. Work is in progress to demonstrate that the RI of the genomic RNA contains nacent subgenomic mRNA strands.

*In collaroration with S. Astier-Manifacier (CNRA, Versailles, France).

R6.33

LEVELS OF VIRAL RNAs AND CAPSID PROTEIN ANTIGENS OF TWO TOMATO BLACK RING VIRUS STRAINS IN CROSS PROTECTED CHENOPODIUM QUINOA. C. Bretout, T. Candresse, M. Ravelonandro and J. Dunez. INRA, Pathologie végétale, BP 131, 33140 Pont de la Maye, France.

Tomato black ring virus (TBRV), a nepovirus, has a bipartite, single-stranded, positive sense RNA genome. In Chenopodium quinoa, a mild strain (TBRV-GC) can protect against apical necrosis caused by a serologically distinct severe strain (TBRV-S). The replication of both strains were monitored in cross protected and singly infected C.quinoa plants by measuring their level of coat protein antigens and RNA during the course of the cross protection trial. ELISA tests showed that sharp differences in levels of the coat protein antigens of both strains were not observed in cross protected plants, which suggested that replication of the severe strain was not affected by the presence of the protecting strain.

In paralell tests, levels of RNAs 1 and 2 of each strain were determined by molecular hybridization using cloned cDNA probes which were specific for each RNA species of each strain. Our results showed that the level of RNA2 of the severe strain (S) was not much affected in cross protected plants but that the level of RNA1 of S was strongly and durably reduced (10 fold).

We are currently using single-stranded RNA probes to refine this analysis by quantifying ds and ss negative sense RNAs. These results will be discussed and compared to other cross protection models.

R6.34

SIMILARITIES AND DIFFERENCES AT THE 3' END REGIONS OF THE GENOMES OF PAPAYA MOSAIC VIRUS AND OTHER MEMBERS OF THE POTATO VIRUS X GROUP. M.G. AbouHaidar. Department of Botany, Faculty of Arts & Science, University of Toronto, Toronto, Canada.

Papaya mosaic virus (PMV) is a potex virus with a single-stranded positive RNA genome. Large portions of the genome have been cloned as cDNA. The nucleotide sequences of cDNA clones corresponding to the 3'OH ends of PMV-RNA as well as that of another potex virus, clover yellow mosaic virus (CYMV) have been determined and compared to that of potato virus X (PVX). The three genomes contain, at their respective 3' ends, a poly(A) tail, a non-coding region and an open reading frame, giving rise to three proteins, which have been identified as correspondig to the respective capsid proteins of these viruses. Some regions of these proteins are highly conserved, reflecting structural similarities and/or evolutionary differences between members of this group of viruses. Furthermore, the PMV genome contains similar nucleotide sequences at the 3' ends of both positive and negative strands.

R6.35

THE STRUCTURES OF POTATO VIRUS X (PVX) AND BARLEY STRIPE MOSAIC VIRUS (BSMV) DOUBLE-STRANDED RNAs. V.V.Dolja and D.P.Grama. Department of Virology, Moscow State University, Moscow 119899, USSR

It was demonstrated that the 3'-terminal poly (A) tail (150-250 nt)in PVX genome and the internal poly(A) tract in BSMV genome had the equivalent poly(U) counterparts in the (-) strands of replicative form (RF) RNA. This finding testifies to the template copying of the poly(A) sequences.

The 3'-termini of the genomic-size (-)strands of both viruses contain an unpaired G. It is possible that the extra G participates in reinitiation of (-) strand synthesis. In contrast to this, the (-) strands of subgenomic (sg) RNAs do not carry the extra Gs. This observation implies that (-) strands of sgRNAs can no longer serve for making new copies of sgRNAs.

The (+) strands of BSMV RFs lack the 3'-ultimate A typical for the encapsidated RNAs. The non-template addition of the 3'-terminal nucleotides can be involved in regulation of the balance of (+) and (-) strand synthesis. Since the RF molecules isolated from the infected cells carry the unpaired nucleotide only in the (-) strand, they serve, possibly, only for the (+) strand synthesis.

R6.36

IN VITRO TRANSLATION OF SWEET CLOVER NECROTIC MOSAIC VIRUS RNA IN RABBIT RETICULOCYTE LYSATE. H.R. Pappu and C. Hiruki. Department of Plant Science, University of Alberta, Edmonton, Alberta T6G 2P5, Canada.

Sweet clover necrotic mosaic virus (SCNMV), a member of the dianthovirus group, is a polyhedral virus containing single stranded bipartite genomic RNA. Translation of the two genomic RNAs (RNA-1 and RNA-2) of SCNMV in rabbit reticulocyte lysate resulted in the synthesis of three major polypeptides, 39K, 36K and 34K. Maximum incorporation of labeled methioning was observed at concentrations of 120 mM K^+, 0.5 mM Mg^{2+} and 200 µg/ml of total genomic RNA. Increasing concentrations of the RNA led to the appearance of a 20K product in certain experiments. The 39K product was selectively immunoprecipitated by anti-SCNMV serum and was identified as the coat protein of the virus by its relative electrophoretic mobility. Results from translation experiments using fractionated RNA-1 and RNA-2 suggest that the 39K product was encoded by RNA-1 of the virus. Investigations on the origin and function of 36K and 34K are in progress.

R6.37

EFFECT OF SUPPRESSOR tRNA ON THE TRANSLATION OF TOMATO MOSAIC VIRUS(ToMV) RNA AND ITS INHIBITION ON VIRUS MULTIPLICATION IN TOBACCO. X-F. Wang, F-Z. Li, P. Tien. Department of Virology, Institute of Microbiology, Academia Sinica, Beijing, People's Republic of China.

The suppressor tRNAs were isolated from temperature mutant SUP6-1 of Saccharamyces cerevisiae containing SUPtRNA (tyr), SUPtRNA(leu) and SUPtRNA(his) and from spinach plant. Both tRNAs could promote the production of 183K readthrough protein product of ToMV RNA in rabbit reticulocyte lysate translational system.

The effect of SUP6-1 tRNAs on multiplication of ToMV in tobacco was studied by both local lession and systemic infection hosts. The lession number on multiplication assay half leaves of N. tobaccum var. Xanthi nc. treated with 60ug SUP6-1 tRNAs each were 35% as many as that of treated with water. The result of local lession test with whole leaves showed that the lession numbers of leaves treated with 60, 120, 240ug of SUP6-1 tRNAs were 49, 24,20% as many as that of treated with water. In the test of systemic infection virus titers were measured by protein A sandwich Elisa at intervals from 28 hr to 23 days after inoculation. The titers in systemically infected leaves of N. tobaccum treated with SUP6-1 tRNAs were 40-80% lower than that of water treated ones.

R6.38

CLONING AND NUCLEOTIDE SEQUENCE DETERMINATION OF POTATO VIRUS X RNA. S.Yu.Morozov, L.I.Lukasheva, B.K.Chernov, K.G.Skryabin and J.G.Atabekov. Department of virology, Moscow State University, Moscow 119899, USSR.

Double-stranded cDNA copies of the RNA of potato virus X (PVX) were inserted into pBR322 by the linker method. The extended 3'-terminal nucleotide sequence of the PVX RNA contained the four open reading frames (ORF's). The first ORF terminates with a single UGA codon at position 1292 from a 3' poly(A). The ORF's 2, 3 and 4 correspond to the regions 1286-945, 1009-800 and 786-76, respectively. The proteins predicted from ORF2 and 3 possess some properties of membrane-associated polypeptides. Considerable amino acid sequence homology exist between the predicted ORF 2 protein and those coded by the RNA2 of hordeiviruses and furoviruses. The ORF4 encodes the PVX capsid protein which is translated from a subgenomic RNA starting at position 812. Unexpected amino acid sequence homology between the PVX capsid protein and the coat proteins of the potyviruses have been observed. The restriction map of the overlapping cDNA copies of the complete PVX genome was determined using the direct method and the partial sequence data.

R6.39

Tomato Yellow Top Virus Relationships.
S. Hassan, Dept. of Plant Pathology, Agri. Uni. Peshawar, Pakistan & P.E.Thomas, IAREC, Prosser, WA 99350, USA.

Serological relationships between Tomato Yellow top virus (TYTV) a luteovirus, and a number of ther luteoviruses were studied. In infectivity neutralization tests antisera against Florida yellow top virus (FYTV), P-4 (BWYV) and PLRV-1 competely eliminated TYTV and PLRV antigens both in scanning pattern and in the normal TYTV bearing zones. Antisera against P-2 and R-Y-I-R (BWYV) partially neutralized TYTV antigens.

In gel-diffusion preciptin tests TYTV antigens reacted strongly with antisera to BWYV, FYTV, PLRV, & TYTV from Australia & New Zealand, wea kly reacted with antisera to Pea leaf roll virus (PeLRV) an.. Tobacco necrotic dwarf virus (TNDV); and did not react with antisera to Barley yellow dwarf virus (BYDV), Soybean dwarf virus (SDV), and carrot red leaf virus (CRLV).

No relationship between TYTV and isolates of BWYV derived from sugar beets and raddish was detected in direct ELISA (DAS). In indirect ELISA TYTV, reacted with antisera to BWYV, PLRV & TYTV from Australia & New Zealand, but did not react with antisera to BYDV, SDV, CRLV & TNDV.

Infectivity neutralization was highly sensitive,double antibody sand witch (DAS) was extremely strain specific, while gel diffusion and indirect ELISA had a broad specificity. Serological results were also substantiated with critical host range, cross protection and matual exclusion tests.

R6.40

Electrophoretic Characterization of Tomato Yellow Top Virus and Potato Leaf Roll Virus.

Sher Hassan, NWFP Agricultural University Peshawar, Pakistan & P.E. Thomas, USDA, ARS, Irrigated Agricultural Research & Ext. Prosser WA 99350, USA.

Cellulose acetate strip electrophoresis technique was used to characterise Tomato yellow top virus, potato leaf roll virus and to investigate the infectivity of the electrophoresed virus. Great variations in rate and pattern of migration was found among TYTV isolates and PLRV at various PHs ranging from 4.5 to 8 in 0.01 M phosphate buffer. Infectivity of electrophoresed virus was not completely destroyed but was partially neutralized. This technique can be successfully used for the separation and diagnosis of different viruses and strains from mixed infection, for the establishment of relationship between viruses and virus strains and for the determination of purity of a virus preparation.

R6.41

SYNTHESIS OF DISEASE-ASSOCIATED PROTEINS IN VIROID-INFECTED TOMATO LEAVES. A. Hadidi.
Plant Genetics And Germplasm Institute, Agricultural Research Service, U.S. Department of Agriculture, Glenn Dale, MD 20769, USA.

Comparison of protein synthesis from uninfected and potato spindle tuber viroid (PSTV)-infected tomato leaves showed that three proteins of 14 kDa, 33 kDa, and 90-100 kDa were enhanced in infected tissue. The 33 kDa protein was the most prominent. These proteins were distributed in the soluble subcellular fractions. The disease-associated proteins were not present at detectable levels in the nuclear or other particulate fractions.

A 33 kDa protein that bound PSTV in vitro was present at detectable levels in the soluble fractions from infected but not uninfected cells. Both the infected and uninfected soluble fractions also contained PSTV binding proteins of 52-55 kDa, 35 kDa, 31-32 kDa, and 16-20 kDa.

R7.1

Molecular characterization of a family of actinophages of *Faenia rectivirgula*.

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Six temperate phages of the thermophilic actinomycete *F. rectivirgula*, one of the agents causing hypersensitivity pneumonitis, where shown to be closely related by means of restriction mapping and cross-immunity testing. Furthermore two virulent phages - one of them originally isolated for *Streptomyces erythraeus* - have genomes related to the temperate phages as shown by restriction mapping and Southern hybridization. Both virulent phages cannot be propagated on strains which are lysogenized with one of the temperate phages. A third virulent phage which exhibited only partial homology with the other phages can be grown on lysogenic as well as non-lysogenic strains of *F. rectivirgula*.

In this paper we discuss the phylogeny of the phages and present our studies on the repression of the virulent phages by related prophages.

R7.2

GENOMIC ORGANIZATION IN MYCOBACTERIOPHAGES I1, I3, I5 AND I8

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Based on restriction endonuclease clevage patterns, dot blot hybridization and Southern blot hybridization analysis, the genomic DNAs of mycobacteriophages I1, I3 and I5 appear to be homologous and undistinguishable but entirely different from phage I8. the genomic double stranded DNA of phage I3 when denatured with alkali, heat, formamide or dimethylsulfoxide breaks down to heterogeneous-sized single stranded (SS) fragments smaller than the intact genome length due to the presence of random SS interruptions on both the strands. The interruptions are in the form of short gaps about 10 nucleotides long. There are 13 to 14 such gaps per DNA molecule. These gaps have no adverse effect on the phage infectivity or DNA transfectivity and they facilitate the compaction and packaging of the DNA by reducing the rigidity of the back bone structure of this GC rich DNA. In contrast, the I8 genome does not harbour any interruptions.

R7.3

AN UNSTABLE LYSOGEN LW INFECTED WITH Cf16-VARIANT. H. DAI
S. Huang. Institute of Botany, Academia Sinica, Nankang, Taipei, Taiwan

In the course of our study in the neolysogenization of filamentous phage Cf16 and it's stable lysogens in Xanthomonas citri[1], we have discovered a new unstable lysogen, LW.

LW lysogen is manifested by the spontaneous appearance of four distinguishable types of phage carrier state, each with a difference phage producing capacity and colony morphology. The phage released from LW, Cf16-v1 produces clear rather than turbid plaque as phage Cf16.

In contrast to Cf16, Cf16-v1 is unstable in the host cell. Only 2.3% of Cf16-v1 infected cells retain the phage genome and go through the neolysogenization process as compared to 92.2% for the Cf16 infected cell.

Recently we have demonstrated that several DNA segments of native plasmid of the host showed homologous to Cf16 genome. Moreover, changes of the DNA organization of the native plasmid are readily detectable according to the infectious status of the host.

This results suggest that (1) a transposable element may be involved in this unstable lysogen system; (2) Cf16-v1 may be resulted from a mutation of Cf16 genome caused by this transposable element; (3) The mutation in Cf16 often results in the nullification of it's ability to undergo neolysogenization in the host cell.

1. Dai,H., Tsay,S-H.,Kuo,T-T., Lin,Y-U, Wu,W-C (1987) Virology (in press).

R7.4

GENETIC TECHNIQUES FOR MAPPING FUNCTIONAL DOMAINS OF ΦX174 NUCLEOCAPSID. J.K. Tuesch*, L. Ilag#, & N.L. Incardona#.
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Genetic techniques have been developed for selecting mutations that lead to defects in nucleocapsid functions of phage ΦX174, i.e. binding and initiation of DNA injection. For *cΔ* mutants, an enrichment step based on loss of infectivity during the eclipse period is followed by a screen for the *cΔ* phenotype. DNA sequencing and eclipse kinetics on 13 mutants have defined three different amino acid replacements in a 17 amino acid residue region in the center of the major capsid protein (F) primary structure. Spontaneous revertants of one *cΔ* mutation behave like suppressor mutations in backcrosses with *wt*. The segregation ratios suggest intragenic suppression for some of these pseudorevertants. One possible role for this domain in ΦX DNA injection is the site for a conformational change induced by virus binding to host receptors. This would result in dissociation of H spike proteins and DNA from the capsid as the genome transfer begins at the *E. coli* lipopolysaccharide receptor sites.

Another class of mutants was selected by first screening a mutagenized population of phage for the *tΔ* phenotype. Each mutant was then subjected to a temperature shift during growth to distinguish between defects in early and late functions. About 10% of these *tΔ* mutants exhibit altered eclipse kinetics at the nonpermissive temperature.

R7.5

DNA/DNA HYBRIDIZATION AND CHARACTERISTICS RELATED TO DNA STRUCTURE AS CRITERIA IN CLASSIFICATION OF BACTERIAL VIRUSES. **V.N.Krylov, Institute for Genetics of Microorganisms, Moscow, U.S.S.R.**

Difficulties in bacteriophage classification are caused by primitiveness of available phenotypic features. Unconditional criterion of relatedness (formation of viable hybrids) can be applied rearily. From preconditions for hybrid formation (similarity in genetical organizations, some DNA homology, functional and structural compatibility of gene modules) only the level of DNA homology can be estimated in a simple way. Nevertheless,the fact of DNA homology itself can not be a crucial evidence of relatedness, as the absence of manifested DNA homology can not be taken as a final evidence of unrelatedness. There is no way to make *a priori* universal decision what DNA homology level and its distribution are necessary to conclude on relatedness. For phages of the same host some additional structural features (genome organization, similar operators, endonuclease sensitive sites) can be useful.

These considerations on the role of DNA features in taxonomic decision will be discussed on examples of lambdoid phages of E.coli, transposable phages of Pseudomonas aeruginosa and a group of phages active on Brevibacterium flavum.

R7.6

WITHDRAWN

R7.7

PHOTOREACTIVATION OF ULTRAVIOLET IRRADIATED RNA PHAGE BY BACTERIA. B. H. Wu, T. Y. Liu, Department of Virology and Molecular Biology, Wuhan, Hubei, People's Republic of China.

The RNA phage Qβ was used to investigate the capability of bacteria to repair ultraviolet (UV) induced damage in the viral RNA genome. When assayed by the formation of plaques, the survival of phage was found to decrease linearly with increased ultraviolet exposure. The infection capacity of UV-irradiated phage was slightly restored after exposion the infected bacteria to photoreactivation condition (white fluorescent), and was not restored in UV-enhanced reactivation condition (far ultraviolet radiation).

The antibiotic rifampicin, which inhibits DNA-dependent RNA polymerase, added 7 minutes after Qβ infection, allows almost normal growthof Qβ, but inhibits photoreactivation of Qβ phage by the host cells. Therefore Photoreactivating enzyme may be induced by UV-damaged RNA phage infection, and produced after 7 minutes post infection.

R7.8

Regulation of Transcription of the Xp10 Genome in Bacteriophage-Infected Xanthomonas campestris pv. oryzae. T.T. Kuo, Y.D. Liao. Institute of Botany and Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan, China

Results of in vivo studies showed the transcription of the Xp10 genome in Xp10-infectedcells shifted from rifampin sensitivity to rifampin resistance. Results of in vitro studies showed that a rapid reduction of rifampin-sensitive RNA polymerase activity coincided with a rapid increase of rifampin-resistant RNA polymerase activity in cell extracts with time after infection. Host and Xp10-encoded RNA polymerases were purified, and the transcripts from these two enzymes were hybridized to the restriction fragments of Xp10 DNA. The RNA probe generated by host RNA polymerase hybridized strongly to the left-most 25% of Xp10 DNA and weakly to the rightmost 75% of Xp10 DNA. The RNA probe generated by Xp10 RNA polymerase hybridized strongly to the rightmost 75% of Xp10 DNA and weakly to the leftmost 25% of Xp10 DNA. Studies with ^{32}P-labeled RNA isolated at various intervals after infection did not reveal any evidence for early versus late differences in transcription.

R8.1

PROTECTION OF NEONATAL FERRETS AGAINST INFLUENZA VIRUS FOLLOWING MATERNAL IMMUNIZATION. C.Sweet, K.Jakeman, D.M.Coates, H.Smith. Department of Microbiology, The University of Birmingham, Birmingham B15 2TT, U.K.

Infection of 1-day-old neonatal ferrets with a virulent influenza virus is invariably fatal, neonates dying of pneumonia or obstruction of the upper airways with a syndrome akin to cot-death.

Such neonates were passively protected following maternal immunization by live infection or with formalin-inactivated virus vaccine. Protection was antibody-mediated and milk-derived. The level of protection with killed vaccine varied from partial to complete and depended upon the number of doses used to vaccinate, the presence of adjuvant, whether mothers were "primed" by prior infection with a serologically heterologous type A virus, and the age of the neonate at challenge. Neonates were completely protected up to 2 weeks of age, but susceptibility returned to nasal epithelium at 5 weeks and to lung at 7 weeks. Mothers immunized up to 9 months previously also partially or completely protected their offspring, this correlating with maternal serum haemagglutination-inhibiting antibody titre at the time of neonatal challenge, not the duration of immunity.

R8.2

VIRUSES ASSOCIATED WITH ACUTE RESPIRATORY INFECTIONS IN AMBULATORY GUATEMALAN PRE-SCHOOLERS. J.R. Cruz, P. Cáceres, F. Cano, A. de Fernández, F. Peralta. Program of Infection, Nutrition and Immunity, Division of Nutrition and Health, Institute of Nutrition of Central America and Panama, Guatemala City, Guatemala.

With the purpose of determining the epidemiology of acute respiratory infections (ARI) among children under five years of age living in marginal urban areas, 200 families from Guatemala City were kept under surveillance from Jan. 1985 until Dec. 1986. Weekly home visits were made by field workers. Children with signs and/or symptoms of ARI were referred to a local health post where a nasopharyngeal aspirate was obtained for viral isolation. During the course of the study a total of 849 specimens were collected and inoculated into duplicate cultures of MDCK, LLC-MK2, HEp-2 and MRC5 cells. Viral agents were isolated from approximately 73% of the samples: 53% of the total were enteroviruses; 10% were CMV; 9% were Rhinoviruses, 8% were identified as adenoviruses, 8% as influenza viruses A and B, 7% as para-influenza 1 or 3 and 4% were RSV. The isolations of entero and rhino viruses were higher during the rainy season (May-Oct) while adenoviruses (types 2,1,5,6,3 & 7) were isolated only in the dry months. Influenza viruses (A/Mississippi/1/85, A/Thailand/10/85, B/USSR/100/83, B/Minnesota/1/85, B/Ann Arbor/1/86, B/Louisiana/1/86) showed no particular pattern. Severe diseases seemed to be associated with mixed infection.

R8.3

INHIBITION OF INFECTIVITY OF RESPIRATORY SYNCYTIAL VIRUS (RSV) VIA MODULATION OF CELLULAR PROTEIN KINASE C (PK-C) ACTIVITY. Y. Okamoto, J.C. Cunella, and P.L. Ogra, Department of Pediatrics, Faculty of Medicine, State University of New York, Buffalo, USA.

Calmodulin (CaM) and PK-C play a central role during cell activation for many cellular processes in a variety of tissues. We evaluated the effect of two selective PK-C antagonists, 1-(5-Isoquinolinylsulfonyl)-2-methylpiperazine (H-7) and Polymyxin B (P-B) on viral infectivity. Pre-incubation of HEp$_2$ cell culture monolayers with H-7 for 5 min. resulted in significant plaque reduction. Maximal inhibition of viral replication (40%) was obtained with highest concentration (200 nM) of H-7. The inhibitory effect was reversible and the infectivity of the virus was reestablished upon removal of H-7. In contrast to the results obtained with H-7, P-B had no inhibitory effect on viral infectivity. Preincubation with W-7 a naphthalene-sulfonomide antagonist of both CaM and PK-C resulted in similar reduction in viral plaque formation. Maximal inhibitory effect of W-7 was 40% and observed with the highest concentrations used (100 nM). Inhibition of CaM with W-7 did not result in further plaque reduction than observed after inhibition of PK-C alone with H7. These results indicate that intermediary steps in RSV replication or attachment may involve CaM or PK-C and that these compounds may be useful as probes to explore mechanisms of viral replication.

R8.4

THE STUDY OF THE HUMORAL IMMUNE RESPONSE TO RHINOVIRUS INFECTION IN SERUM AND NASAL SECRETIONS USING AN ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA). W. S. Barclay, W. Al-Nakib, D. A. J. Tyrrell, MRC Common Cold Unit, Salisbury, Wiltshire, England.

This study describes the first enzyme-linked immunosorbent assay (ELISA) capable of detecting class-specific immunoglobulin responses to rhinovirus infection in human sera and nasal secretions. The ELISA utilizes a rabbit antirhinovirus hyperimmune serum as a capture antibody. The ELISA detects rhinovirus specific antibody in the serum at dilutions of $1/10^6$ and $1/10^{3.5}$ for IgG and IgA immunoglobulins respectively. It is thus 10^2 - 10^4 times more sensitive than our standard neutralization test, allowing a reliable detection of rhinovirus-specific local antibody in unconcentrated nasal washings which might be particularly relevant for protection against rhinovirus reinfection. Indeed, a preliminary study of twelve volunteers challenged with HRV-EL has indicated that IgA antirhinovirus antibodies in both serum and nasal secretions correlate better with protection against reinfection than do those of the IgG class.

Details of these and other data on homotypic and heterotypic responses following rhinovirus infection in man will therefore be presented.

R8.5

EFFECT OF RELATIVE HUMIDITY ON THE SURVIVAL OF HUMAN CORONAVIRUS 229E ON NON-POROUS INANIMATE SURFACES. S.A. Sattar, Y. Karim, C.M. Johnson-Lussenburg. Department of Microbiology and Immunology., School of Medicine, University of Ottawa, Ottawa, Canada K1H 8M5.

Coronaviruses cause respiratory and enteric diseases in humans and animals. Virus survival on environmental surfaces was studied to assess their potential as vehicles for coronavirus infections. Stainless steel disks (1 cm diameter) were chosen to represent such surfaces. Each disk was contaminated with 10 uL (approximately 100,000 plaque forming units) of the virus suspended in 5 mg/mL of bovine mucin in normal saline. The inoculum was first allowed to dry in a laminar flow hood for 1 h under ambient conditions. Tryptose phosphate broth was used to elute virus from the disks, and eluates plaque assayed in L-132 cells. There was no detectable loss in virus titre during the initial period of drying. The disks with the dried inoculum were then placed at 20±1°C under either low (20±5%), medium (50±5%) or high (80±5%) relative humidity (RH) level. Virus survival was studied for 24 h and the coefficient of virus inactivation (Ki) was determined as $\log_{10}$ loss in virus titre/h. The Ki values for the virus at the high, medium and low RH were 0.30, 0.10 and 0.07, respectively, suggesting a role for environmental surfaces as vehicles. The capacity of the virus to survive well at the low RH may also explain the seasonal nature of human respiratory coronavirus outbreaks.

R8.6

OCCURRENCE OF RSV SUBTYPES A AND B STRAINS IN JAPAN. H. Tsutsumi, M. Onuma, K. Suga, Y. Chiba, S. Tsugawa, T.Nakao and P. L. Ogra. Department of Pediatrics, Sapporo Medical College, Sapporo, Japan, and Department of Pediatrics and Microbiology, SUNYAB, Buffalo, New York, USA.

The subtype characteristics of 23 wild strains of respiratory syncytial virus(RSV) isolated in Sapporo, Japan during a time period of 8 years(1980-1987) were determined by the use of monoclonal antibodies(MAbs) raised against RSV Long strain. Six MAbs to large glycoprotein(G) and 8 MAbs to fusion protein(F) were used in immunofluorescence and radioimmunoprecipitation assays. Two major subtypes and two intermediate types of the virus were demonstrated. Subtype A included 16 virus isolates from every seasons. Subtype B included 6 virus isolates from 1983 and 1985. One intermediate type(A') was isolated in 1980 and other(A") was isolated in 1983. Subtype A viruses reacted with all the MAbs. On the other hand, subtype B viruses reacted with none of anti-G MAbs and 6/8 anti-F MAbs. Subtype A' and A" reacted with 5/6 and 3/6 anti-G MAbs respectively, and both reacted with all anti-F MAbs. The 4 anti-G and 2 anti-F MAbs employed to characterize 4 subtypes have neutralizing activity. These observations suggest that the altered epitopes demonstrated by these MAbs contain some determinants involved in virus infectivity and may explain alternate and concomitant occurrence of these RSV subtypes infection and previously observed strain variation in neutralization test.

R8.7

STUDIES OF ACQUISITION OF ANTIBODIES TO CYTOMEGALOVIRUS FOLLOWING ALLOGENEIC BONE MARROW TRANSPLANTATION.

P.D. Griffiths, J. Wimperis*, M.K. Brenner*, J-P. Grob*, J.E. Grundy, H.G. Prentice*. Departments of Virology and Haematology*, Royal Free Hospital School of Medicine, London NW3 2PF, England.

Weekly viral surveillance cultures of urine, saliva and blood were used to identify patients with CMV infection post transplant. Those without active infection were studied serially post transplant to determine whether CMV antibodies were derived from the donor or recipient. 11 seronegative recipients remained seronegative up to one year even when their donors had been seropositive. In contrast, 8 seropositive recipients remained seropositive post transplant even when their donors had been seronegative. We conclude that antibodies are produced post transplant by donor lymphocytes which encounter CMV antigens persisting within the recipient.

Initially seropositive recipients who did excrete CMV post transplant were next analysed. Among 13 patients with seronegative donors 9 excreted CMV of whom 8 developed pneumonitis. In contrast, of 26 patients with seropositive donors 13 excreted CMV but only 4 developed pneumonitis. We conclude that, even after T-cell depletion of marrow, seropositive donors can provide some protection against CMV disease for seropositive recipients and suggest this is due to transfer of immuno-committed B-lymphocytes.

R8.8

CHARACTERIZATION OF MOUSE T CELL LINES SPECIFIC FOR RESPIRATORY SYNCYTIAL VIRUS (RSV) NUCLEOPROTEIN AND FUSION PROTEINS.

M.J. Cannon[1], J.R. Rothbard[2], B.A. Askonas[1]. [1]Division of Immunology, National Institute for Medical Research, The Ridgeway, London NW7 1AA, UK and [2]Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX.

RSV-specific T cell lines were established from BALB/C mice primed with vaccinia virus recombinants expressing the fusion (F) and nucleoprotein (N) genes of RSV A2. The F-specific line was $Lyt2^+$ and cytotoxic, but the N-specific line possessed $Lyt2^+$ and $L3T4^+$ T cells and exhibited both cytotoxic (Tc) and helper (TH) T cell functions.
N and F-specific Tc strongly lysed target cells infected with RSV A2 or Long (RSV serogroup A), but showed minimal lysis of targets infected with serogroup B strains, notably 8/60 and 18537. In contrast, the N-specific TH response was broadly cross-reactive for serogroups A and B, suggesting divergent Tc and TH repertoires for N-specific cellular responses in mice primed with the N-vaccinia recombinant virus.
Tc and TH fine specificity is being mapped with the aid of synthetic peptides representing candidate T cell epitopes on these proteins.

R8.9

SUSCEPTIBILITY OF RSV-INFECTED HUMAN MONOCYTES TO CELL-MEDIATED CYTOTOXICITY REACTION.

Y. Chiba, K. Suga, Y. Higashidate, K. Honjo, H. Tsutsumi, T. Nakao and P. L. Ogra, Department of Pediatrics, Sapporo Medical College, Sapporo, JAPAN, and Department of Pediatrics and Microbiology, SUNYAB and Buffalo Children's Hospital, USA.

Human peripheral blood monocytes cultured by type AB serum were infected with respiratory syncytial virus (RSV) and the virus specific antigen was detected on the cell surface. Employing these RSV infected and ^{51}Cr labeled monocytes as target cells, we established a system for cell-mediated cytotoxicity test. Thus, lymphocytes exhibited 30 to 60% of specific cytotoxic activity to RSV-infected autologous monocytes in the presence of specific antibody. Furthermore, lymphocytes obtained from infants with a recent RSV infection and cultured with UV inactivated RSV in vitro induced cytotoxic activity (up to about 20%) to such autologous RSV infected target cells. This response, however, was not observed in similarly cultured lymphocytes of normal newborn infants. It is suggested that this system is useful for assaying RSV-specific T cell-mediated cytotoxic response which may require identity of the major histocompatibility complex (MHC) between effector cells and target cells.

R8.10

Development of an immunoassay using recombinant DNA-derived nucleoprotein (rD-NP) of influenza type A virus and monoclonal antibody to human IgG for a class specific antibody assay for influenza virus.

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Influenza A nucleoprotein (NP) derived from the full length cloned gene expressed in E. coli was evaluated in a solid phase EIA for influenza antibody. Monoclonal antibody to human IgG was used for detection. Direct and Indirect assays were developed and sera were tested in serial- and single-dilution formats. Preliminary results indicated rD-NP and virion-derived NP antigens were comparable. Thirty-two paired sera from influenza A infected patients were tested. The most sensitive assay (indirect, serial dilution) detected 30 (94%) rises and the simplest assay (direct, single dilution) detected 26 (81%) rises, compared to 25 (78%) for CF and 27 (84%) for EIA with whole virus antigen. Sera from 18 control patients were negative in all assays. Fifteen paired sera from influenza B infected patients were negative except for 1 borderline positive by the direct-single dilution assay. These results indicate modern, automatable serodiagnostic tests for influenza can be developed with reagents derived through rDNA techniques, assuring consistent performance.

R8.11

FAILURE OF PRIOR NATURAL INFECTION TO PROTECT AGAINST INFECTION WITH INFLUENZA A/PHILIPPINES (H3N2) VIRUS.

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Longitudinal studies of persons in the Houston Family Study revealed that 81 of 222 persons were infected with influenza A/Bangkok (H3N2) in 1980-81. Only 6 (7.4%) of 81 were reinfected with A/Bangkok when this virus returned in 1982-83; while 44 (31.2%) of 141 persons who escaped infection in 1980-81 were infected in 1982-83. The protective efficacy of prior natural infection was 76.3%.

Including children born after the 1980-81 epidemic, 53 persons were infected with A/Bangkok for the first time in 1982-83; 13 (24.5%) were reinfected with the new A/Philippines (H3N2) variant in 1984-85. This rate was not different than the rate of 34.7% for 101 persons who were not infected previously with A/Bangkok. The protective efficacy of prior infection with A/Bangkok was only 29.4%.

Illnesses associated with A/Philippines infection were as frequent and as severe among persons who were infected earlier with A/Bangkok as among persons who were not infected previously with A/Bangkok.

Protection against influenza A (H3N2) infection appeared to be variant-specific during this period. Earlier observations in this population had shown protection against A/Bangkok by prior infection with the A/Texas variant in 1977-78.

R8.12

VIRUSES ISOLATED FROM THE RESPIRATORY TRACT OF CATTLE IN POLAND.

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The diseases of the respiratory tract of calves are a great problem in large farms in various regions of Poland. Twelve strains of cythopatogenic viruses were isolated on the primary and secondary calf kidney call cultures from the nasal and ocular swabs from 56 calves with the symptoms of respiratory tract inflammations.

Six strains were classified physico-chemically and serologically to Herpesviridae as Bovid herpesvirus 1. Two strains were classified to Togaviridae as Bovine diarrhoea mucosal disease virus, and four were antigenically identic with Bovine parainfluenza 3 virus, strain SF 4. It is suggested that the isolated viruses are a primary cause of enzootic respiratory diseases in calves.

R8.13

SYNERGISTIC ROLE OF STAPHYLOCOCCAL PROTEASE IN THE DEVELOPMENT OF INFLUENZA PNEUMONIA. M. Tashiro, H.-D. Klenk, R. Rott. Dept. Bacteriol., Yamagata Univ. Sch. Med., Yamagata, Japan, Inst. Virol., Univ. Giessen, Giessen, FRG.

Several strains of Staphylococcus aureus were found to secrete trypsin-like proteases that activate infectivity of influenza virus by proteolytic cleavage of the hemagglutinin (HA). The HA of an individual virus strain could be cleaved by the proteases of some but not all staphylococcal strains, and a given enzyme could cleave only some but not all HA analyzed.

When mice were coinfected intranasally with the appropriate strains of influenza virus and Sta. aureus, the HA was readily activated allowing multiplication of virus in the lung. Under these conditions, the animals came down with a fatal disease exhibiting extended lesions in the lung tissue. In contrast, after infection with virus or bacteria alone, there were no significant pathological changes. When leupeptin, which was shown to inhibit the bacterial proteases, was given intranasally to the co-infected mice, development of the lung lesion was suppressed through inhibition of proteolytic activation of the progeny virus in the lung.

These observations show that coinfecting bacteria can play an important role in the development of influenza pneumonia by providing a protease for activation of the HA, and that protease inhibitor could be a therapeutic drug against influenza pneumonia.

R8.14

PROTECTION PROVIDED BY INACTIVATED EQUINE INFLUENZA VACCINES AGAINST EXPERIMENTAL INFECTION OF PONIES WITH INFLUENZA A/EQUINE/NEWMARKET/79 (H3N8) VIRUS. J.Mumford, J.Wood, D.Jessett, D.Hannant, R.F. Cook. Equine Virology Unit, Animal Health Trust, Nr. Newmarket, Suffolk, England CB8 7PN

Clinical signs of equine influenza have been reproduced in ponies in a series of experiments using intranasal instillation or exposure to aerosol as the methods of challenge with influenza A/equine/Newmarket/79 (H3N8) virus. Protection provided by inactivated vaccines of known antigenic content has been evaluated by measurement of antibody responses, virus excretion and clinical signs (febrile responses and coughs) following challenge.

In three experiments using intranasal challenges protection was demonstrated in ponies with circulating antibody levels >65mm^2 (zone size in single radial haemolysis (SRH) test) whereas in three experiments using aerosol challenges protection could only be demonstrated in ponies with SRH antibody levels >120mm^2. In those ponies which were not fully protected a clear relationship could be demonstrated between pre-challenge antibody levels and duration of virus excretion and duration of febrile responses and coughing. Using these parameters, the protection provided by a series of inactivated (whole virus and subunit immune stimulating complexes, ISCOMs) has been evaluated.

R8.15

ANTIGENIC ANALYSIS OF TYPE B INFLUENZA VIRUS. HI TESTS WITH ABSORBED RABBIT ANTISERA AND IMPACT OF PASSAGE HISTORY OF THE VIRUS. Y. Yamazi, H. Suzuki, M. Takahashi (Nippon Med. Sch.,Tokyo), M. Nakayama, A. Oya (NIH, Tokyo), Y. Takeuchi (Chiba Pref. Serum Inst.), Y. Sato (Yamanashi Pref.Inst.) K. Nakamura (Nagano Pref.Inst.),Japan;B.Pongprote, S. Suprasert, J. Supawadee (Chiang Mai Univ.) Thai.

Type B influenza virus prevailed in Japan and North Thailand in the winter or December, 1985. Thirty strains isolated in the season were tested by HI tests with rabbit antisera raised to nine representative strains of the virus circulated during 1973 to 1985. Absorbtion of the sera with virus prevailed in 1973 to 1983 facilitated the study of antigenic drift of the virus, and antigenic differences between the type B influenza virus strains, which were not found by the conventional HI tests with ferret antisera raised to representative viruses, were recognized by HI tests with absorbed sera. Absorbed sera showed higher HI titers with viruses grown in MDCK cell cultures than with those in fertile eggs. Antigenic structures of the type B virus isolated in the same season were similar between Thai and Japan.

R8.16

INCREASED FREQUENCIES OF ANTIGENIC VARIANTS OF INFLUENZA A VIRUS GROWN IN THE PRESENCE OF HUMAN LEUKOCYTES.

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Polymorphonuclear leukocyte (PMN) exudation onto the infected mucous membrane are the characteristic feature of influenza in humans or ferrets. As a phagocytic cell, PMN usually generates active oxygens in response to stimulations by infecting microbes. So, mutagenic effects of PMN to replicating influenza A viruses were examined in an in vitro experimental system by using monoclonal antibodies.

Eight hours after inoculation with cloned A/NWS (H1N1) virus, the infected MDCK cells were treated with human PMNs. After subsequent 15-hr incubation at 37 C, the propagated viruses were harvested and examined for frequencies of antigenic variants which formed plaques in the presence of neutralizing anti-HA monoclonal antibodies. Although reductions in viral yields after exposure to PMNs were not recognized, significant increase in the antigenic variations was observed. Such effect by PMN was abolished by addition of superoxide dismutase to the culture.

R8.17

HOSPITAL DISCHARGES IN RESPIRATORY INFECTIONS IN TWO AGE GROUPS IN COSTA RICA. Libia Herrero & H. Vargas.Faculty of Microbiology,University of Costa Rica & Social Security, San Jose,Costa Rica.

Over 90% of respiratory infections are caused by viruses. This work is not an etiological study but an epidemiological description of hospital discharges with a final diagnosis of acute respiratory infections,influenza and pneumoniae. The data were obtained by the computarized system of information on hospital discharges of the Costa Rican Social Security which covers 96% of hospital discharges of this country. Data from 1982 to 1985 and two age groups (less than 2 and over 60 years old) were obtained and distribuited according to month, number of discharges and letality. A well defined seasonality was determined in those infections which attack children, with peaks distribuited during the months of july to december in the four years studied. Seasonality was not found in persons over 60 years old, except in 1983, where a small peak was found during setember and october. Letality was found to be ten times greater in the group of persons over 60 years of age as compared to the group of children. Seasonality was not found in the letality ratios in both groups of age, but a low ratio was found in children during the months of greater discharges.

R8.18

HAEMAGLUTINATION-INHIBITING ANTIBODY RESPONSES AFTER TRIVALENT INFLUENZA VACCINATION IN OLD PEOPLE. R. Ortiz de Lejarazu, T. Pérez, J. Castrodeza, M.A. Bratos, A. Rodríguez-Torres. Influenza Center, Department of Microbiology, Faculty of Medicine, University of Valladolid. Valladolid, Spain.

The haemaglutination-inhibiting antibody responses were measured in a group of 315 old age recipients (mean age 68.5 ± 8.1 years) of a single dose of trivalent inactivated commercial influenza vaccine (A/Philippines/2/82, A/Chile/1/83 and B/USSR/100/83). Pre and post vaccination sera were obtained with a mean interval of 27.9 ± 5.7 days.

Before vaccination, 90% of vaccinees had antibodies ≥ 1/40 against A/Philippines, 87% to A/Chile, and 32% to B/USSR. After vaccination these percentages reached 99%, 100% and 90% respectively.

The geometric mean titre of antibodies increased from 1/104 to 1/274 against A/Philippines, from 1/95 to 1/313 to A/Chile and from 1/8 to 1/47 to B/USSR.

Seroconversion (four-fold increase of antibodies in the second serum) to A/Philippines was found in 110 (35%), to A/Chile in 145 (46%), and to B/USSR in 122 (38.7%) of vaccinees. Results of the heterologous response against 1986-87 influenza virus (A/ Mississipi/1/85, A/Taiwan/1/86 and B/Ann Arbor/1/86) and also the response against ether-extracted B antigen will be discused.

R8.19

TOPOLOGY OF BOVINE HERPES VIRUS-1 (BHV-1) GLYCOPROTEIN gIV. G. Hughes, S. van Drunen Littel-van den Hurk and L.A. Babiuk. VIDO, University of Saskatchewan, Saskatoon, Canada.

A panel of monoclonal antibodies specific for BHV-1 glycoprotein gIV (71K) was derived from mice immunized with affinity purified gIV. Virus neutralization assays have identified 9 monoclonal antibodies able to neutralize BHV-1 infection of GBK cells with and without complement. None of these monoclonal antibodies were able to demonstrate killing in antibody-complement mediated cell lysis assays at dilutions greater than 10^{-1}.

Competition binding assays were performed in order to determine the minimum number of epitopes on gIV. The tests were performed reciprocally using peroxidase linked IgGs and identified three antigenic domains involved in virus neutralization. Antigenic domain 1 comprises 3 overlapping or identical epitopes, 1a, 1b and 1c, similarly domain 3 is made up of 4 identical or overlapping epitopes 3a, 3b, 3c and 3d. Domain 2 is represented by a single epitope 2.

R8.20

VIRAL ETIOLOGY IN ACUTE LOWER RESPIRATORY INFECTIONS (ARI) IN CHILDREN FROM A CLOSE COMMUNITY. G. Carballal, M. Avila, H. Rovaletti, M.C. Weissenbacher. Department of Microbiology. Faculty of Medicine, Buenos Aires and "Noel Sbarra" Hospital, La Plata. Buenos Aires ARGENTINA.

From June 1984 to June 1986, children under five years with lower ARI living in an orfan house and coming from low imcome families were studied. Sixty nine % were under 1 year and 58% males. 204 nasopharyngeal aspirates (NPA) were studied by both a rapid technique - IFI- and isolation in MRC-5, Hep-2, LLC-MK2 and MDCK cells.

NPA cells were stained for RSV,adenovirus, Flu A and B and Para Flu 1 and 3. Clinical entities observed were: bronchitis 46%; pneumonias 24%; bronchiolitis 21% and bronchopneumonias 8%.

Viral etiology was obtained in 21% of cases. From these, 53% corresponded to RSV; 19% to unidentified viruses; 14% to adenovirus; 7% to Flu A; 5% to Para Flu 3 and 2% to RSV plus adenovirus. RSV peaked in winter and was found in 14% of pneumonias, 11% of bronchiolitis and bronchopneumonias and 9% of bronchitis.

R8.21

ISOLATION AND CHARACTERIZATION OF THE INFLUENZA C GLYCOPROTEIN. H. Meier-Ewert and F. Formanowski. Department of Medical Virology, Faculty of Medicin, Technische Universität München, Biedersteiner Str. 29, 8000 München 40, F.R.G.

The surface glycoprotein of influenza C Johannesburg/1/66 was removed from the virion by treatment with the protease bromelain. The isolated glycoprotein has an estimated molecular weight of 75 000 dalton. The calculated molecular weight of the remaining membrane-bound portion of the molecul is 3 000 dalton. The isolated glycoprotein was purified by sucrose density gradient centrifugation. The position of the protein trimer band in the gradient indicated a sedimentation coefficent of approximately 10 S.

After isolation of the uncleaved glycoprotein (gp I), from MDCK-cell grown virus, with bromelain, the protein is cleaved into two subunits held together by a disulphide bond. The estimated molecular weights of the subunits are 59 000 dalton and 28 000 dalton.

Comparison of the cleaved glycoprotein from cell grown virus to gp 65 from egg grown virus, by SDS-polyacrylamidegelelectrophoresis, showed no difference in the migration of the two proteins. This indicates that cleavage of cell grown glycoprotein by bromelain occurs near the natural cleavage site. This bromelain cleavage site was further analysed.

R8.22

AMINO ACID SUBSTITUTIONS IN THE HN GLYCOPROTEIN OF ANTIGENIC VARIANTS OF NEWCASTLE DISEASE VIRUS. R.M. Iorio, R.J. Syddall, R.L. Glickman, J.P. Sheehan and M.A. Bratt. Department of Molecular Genetics and Microbiology, University of Massachusetts Medical School, Worcester, MA, USA.

Nonneutralizable antigenic variants were selected with monoclonal antibodies (MAbs) specific for several sites on the hemagglutinin-neuraminidase (HN) glycoprotein of Newcastle disease virus. Some of the variants selected with MAbs specific for antigenic site 2 demonstrate one or more of the following properties: a) increased neuraminidase activity; b) increased mean embryo death time (a measure of virulence); c) decreased HN content in virions; d) ability to bind selecting antibody without subsequent neutralization; and, e) temperature-sensitivity (making possible the isolation of revertants).

In order to identify the amino acid residues which form this antigenic determinant (as well as others) and the substitutions responsible for the variant phenotypes, we have sequenced, using primer extension and the "di-deoxy" method, selected variant HN's based on the deduced amino acid sequence of the HN of strain AV (T. Morrison, personal communication). The data indicate that the amino acid substitutions in site 2 variants are clustered in a domain 50-60 residues from the carboxy terminus of the molecule. The sequence of site 2 revertants and variants in other sites are presently being determined.

R8.23

UPPER RESPIRATORY DISEASE BY COX. A-21 VIRUS IN PRISON Y. Matsunaga, S. Yamazaki, M. Murao, T. Momose and S. Makino. NIH of Japan, Tokyo; Saitama Inst. Pub. Hlth., Saitama; K. Juvenile Prison, Saitama; Japan

An out break of upper respiratory disease caused by coxsackie virus A-21 (cox.A-21) occurred in one juvenile prison in Saitama, Japan during October to November 1985.

About 1,200 male prisoner aged 18 to 25 were accommodated and an approximately 10% of them were hospitalized during the epidemic. Main symptoms were fever, redness and pain of throat, chill and arthralgia. Most of the patients leave the hospital within 4 days.

Virological examinations were done with 10 patients hospitalized on 17 Oct. Nine virus strains were isolated from throat swabs and eight of them were identified as cox.A-21. Antibody responce was demonstrated in eight of patients by neutralization test.

Seroepidemiological study was done with sera obtained from healthy people living in Saitama prefecture. Before epidemic, antibody was not detected in the sera of age group under 30. Antibody positive percentage rose gradually over thirties, i.e. 30% in thirties, 40% in fourties, 50% in fifties and 100% in over 70.

This is the first epidemic case of cox. A-21 virus infection in Japan. Epidemiological features of outbreak in the juvenile prison are similar to those observed in military recruits. It occurred in autumn, among antibody free young men living closely.

R8.24

RHINOVIRUS SEROTYPES AND ACUTE ASTHMA IN CHILDREN. I. Orstavik, K.-H. Carlsen, The Microbiological Laboratory, Ullevål Hospital, 0407 OSLO 4, Norway.

During a 12 months study period, rhinovirus was isolated from 55 infants and children admitted with acute respiratory disease. Of the patients, 22(40%) had acute asthma.

Forty-nine of the 55 virus strains were available for study and were tested for neutralization against rabbit hyperimmune sera produced against 17 of the virus strains.

Of the 17 serotypes, two serotypes were represented by six virus strains each, four types by four strains each, two types by two strains each, and of the remaining nine types only one strain of each was found. Eight of the 49 rhinovirus strains could not be neutralized by the hyperimmune sera available.

As a rule, the same serotype could be encountered in different seasons of the year. No particular serotype(s) was predominant among the children with acute asthma.

R8.25

ULTRASTRUCTURE MORPHOLOGY OF UNKNOWN VIRUS-LIKE PARTICLES ASSOCIATED WITH PNEUMOENTERITIS IN CATTLE.

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During examination of tissue cultures infected from filtrates of fecal samples and/or lungs, virus-like particles (VLPs) 40 - 60 nm in diameter were observed. The infected cells did not show any fluorescence with bovine virus diarrhoea/mucosal disease virus specific sera. These VLPs were replicating in membrane bound inclusions and accumulated within vesicles in the cytoplasm. Liberation of these particles was probably by exocytosis. The VLPs were variable in diameter and shape with a translucent center and were enveloped.

Negative staining of fecal filtrates and culture supernatants revealed the presence in both of the same morphological VLPS.

R9.1

AVIAN SARCOMA VIRUS 17 AND ITS ONC GENE, JUN. T.J. Bos, T. Nishimura, W. Uckert, H. Tsuchie, and P.K. Vogt. Department of Microbiology, University of Southern California, School of Medicine, Los Angeles, California, U.S.A.

Avian sarcoma virus 17 carries a new putative onc gene, jun. Jun is a cell-derived genetic insert into the retroviral genome, replacing all of the pol and part of the gag and env genes. It is 0.93 kb in length. ASV 17 has been cloned, and the jun insert has been sequenced. The amino acid sequence of jun shows a strong homology in its carboxy terminal portion to the carboxy terminal DNA-binding region of the yeast transcriptional activator protein, GCN4. To a lesser extent jun is also related to fos and myc. The jun protein is localized in the nucleus of the ASV 17-transformed cells and, judged by its homology to GCN4, may also be DNA-binding and may function as an important regulatory protein in the cell. Genomic clones of the cellular jun locus have been obtained from a chicken DNA library. The structure of the cellular jun gene is currently being investigated.

R9.2

POSSIBLE INTERACTION OF A RETROVIRUS AND A LENTIVIRUS IN JAAGSIEKTE, A PULMONARY ADENOCARCINOMA OF SHEEP.
O.W. Verwoerd, A-L Williamson, D.F. York, M.S. Myer and H.F.A.K. Huchzermeyer, Veterinary Research Institute, Onderstepoort, 0110 South Africa.

Jaagsiekte has been closely associated with maedi, a progressive interstitial pneumonia of sheep and goats, in many parts of the world. The two pathological conditions are respectively caused by a retrovirus (JSRV) and a lentivirus, which was shown to co-exist in the lung.

In order to investigate a possible synergism between the two viruses, the effect of lentivirus infection alone and in combination with JSRV was investigated. A mild immunosuppressive effect, mainly on the macrophage component, was demonstrated in vivo and in vitro for the lentivirus. However, immunosuppression was more pronounced in the combined infection, suggesting that both viruses affect the immune system, resulting in a bilateral enhancement.

R9.3

DETECTION OF HTLV-I PROVIRUS AND ANTIBODIES TO HTLV-I IN PATIENTS WITH LYMPHOPROLIFERATIVE DISEASES. R.Kukaine, L.Nagayeva, V.Ose, I.Kalnbērza, M.Murovska, R.Vigele, T.Tkatchenko. August Kirchenstein Institute of Microbiology, Latvian SSR Academy of Sciences, Riga, Latvian SSR, USSR

Patients with various forms of lymphoproliferative diseases were investigated in order to detect HTLV-I infection. Plasmid pMT-2 was used to detect the integrated HTLV-I provirus. Antibodies to HTLV-I were determined by indirect immunofluorescence and ELISA.

We examined patients with preleukemia, with acute and chronic T, B and monocytic leukemias. Among the investigated patients antibodies to HTLV-I and the integrated HTLV-I provirus were detected not only with T-cell leukemia, but with other forms of leukemias as well.

Electron microscopy detected oncoviral particles in patients with T-cell preleukemia and with B-cell leukemia.

The patients under study seldom possessed antibodies to HTLV-I. These antibodies were not detected also in patients with integrated provirus in lymphocyte genome. The absence of antibody reaction testifies to the depression of immune system in patients with lymphoproliferative diseases.

R9.4

STUDIES ON THE REL GENE PRODUCT.
K. Garson and C. Y. Kang, Department of Microbiology and Immunology, Faculty of Health Sciences, University of Ottawa, Ottawa, Canada.

Reticuloendotheliosis virus, strain T (REV-T), is a highly oncogenic avian retrovirus which carries the v-rel oncogene. In order to understand the mechanism by which REV-T transforms cells, the v-rel oncogene product was expressed in a heterologous cell system. V-rel was placed under the control of the SV40 late promoter to allow its expression in Cos 1 cells.

Antisera produced against a rel-specific synthetic peptide was used in a Western blot assay to identify the v-rel oncogene product. The protein expressed in Cos 1 cells was similar in size to the 55 kD protein expressed in REV-T transformed chicken bone marrow cells. In addition, 60% of the v-rel coding region was cloned into pUC 19 in frame with the lac Z gene, and the resulting hybrid protein purified for functional studies. The biochemical activities of the native rel protein, chimeric rel-lac protein and proteins produced from the v-rel gene altered by in vitro mutagenesis will be discussed.

R9.5

IMMUNO ELECTRON AND LIGHT MICROSCOPY OF tsRSV-TRANSFORMED FIBROBLASTS. M.V.Nermut and P.Eason, National Institute for Medical Research, London NW7 1AA, U.K.

We have carried out a correlative light and electron microscopic study of LA-29 cells transformed by ts-mutant of Rous sarcoma virus using both immunofluorescence (of whole cells) and colloidal gold labelling of substratum adherent (ventral) membranes obtained by lysis-squirting (followed by freeze-drying and Pt/C replication).

Within 3 days of culture at permissive temperature cells rounded up leaving behind retraction fibres. In interference reflection microscopy no typical focal contacts but numerous 'point contacts' were observed. About 50% of the latter contained actin and clathrin sheets were seen on small membrane patches in shadowed replicas after heavy squirting. Gold immunolabelling of ventral membranes with monoclonal antibody revealed vinculin at stress fibres termini in normal cells, but on patches of membrane associated particles in transformed cells. After the shift to permissive temperature $pp60^{src}$ and phosphotyrosine containing proteins (using rabbit antibody to synthetic hapten p-azobenzene-phosphonate) were found associated with the inner surface of ventral membranes. The finding of actin in the retraction fibres and the presence of strong points of adhesion (clathrin coated membrane patches) in transformed cells suggest that the primary effect of the oncogene product is at the stress fibre bundles rather than at the actin to membrane association.

R9.6

INSERTION OF SIGNALS FOR AUTOLYTIC CLEAVAGE IN VIRAL cDNAs PROVIDES NEARLY CORRECT 3' ENDS OF VIRAL RNA TRANSCRIPTS.
Josef J. Bujarski and Paul Kaesberg. Biophysics Lab and Dept. of Biochemistry, University of Wisconsin, Madison, Wisconsin 53706, USA.

Transcription of viral cDNAs integrated into the nuclear genome of transgenic plants usually generates viral RNAs containing extraneous sequences at their termini. While the size of these sequences at the 5' end can be reduced relatively easily by reconstruction of the promoter junction it is difficult to avoid some spacer sequences and a polyA stretch at the 3' end. Existence of these extra sequences affects such processes as viral RNA replication or encapsidation.

We are using the autolytic processing activity of sequences found in satellite RNA of tobacco ringspot virus (STobRV RNA) in an attempt to overcome this problem (1). A DNA fragment, isolated from a STobRV cDNA clone (generously supplied by Dr. A. Hampel at Northern Illinois University) and containing all the sequences required for self-cleavage was inserted just downstream from the 3' end of an infectious cDNA clone of brome mosaic virus (BMV) RNA3 (2). Its self-cleavage activity was checked in vitro after transcription with SP6 RNA polymerase.

In cooperation with Drs. D. Merlo and S. Loesch-Fries at Agrigenetics, Madison, WI, we are generating transgenic tobacco plants which would transcribe BMV RNAs and self-regenerate their almost correct 3' ends. We expect that as in nonpolyadenylated mRNAs of histone genes, the removal of the polyA stretch (and most of the other 3' end heterologous sequences), will produce transcripts operational in the cytoplasm. We plan to use this system to analyse in vivo processes of recombination between BMV RNA components without replication and encapsidation and symptom generation without replication.

(1) Prody, G. et al. (1986) Science 231: 1577-1580.
(2) Ahlquist, P. and Janda, M. (1984) Molec. Cell. Biol. 4: 2876-2882.

R9.7

HARVEY MURINE SARCOMA VIRUS TRANSFECTED NORMAL BRONCHIAL EPITHELIAL (NHBE) CELLS PROGRESS TO MALIGNANT GROWTH IN ATHYMIC NUDE MICE AFTER TRANSFORMATION. G. H. Yoakum, L. Malan-Shibley, and C. C. Harris, Laboratory of Human Carcinogenesis, NCI, Bethesda, MD 20892.

Transfection of NHBE cells with vHa ras oncogene led to the isolation of transformed cells (TBE-1) with an indefinite life span. TBE-1 cells contained a subpopulation capable of anchorage independent growth in soft agar (TBE-1SA). The tumorigenic potential of vHa ras transformed NHBE cells was tested in irradiated, Balb/c, athymic nude mice by subcutaneous (sc) injection of TBE-1 and TBE-1SA cells. The TBE-1 cells required inoculations of 2 x 10^7 cells at 2 sites in twenty mice to detect one tumor in 13 months. The TBE-1SA cells were tumorigenic in less than 9 months in over 60% of mice when 1 x 10^6 cells were inoculated at a single site. Human cell lines were recovered from the tumor tissue in each of these experiments, and the tumor tissue identified as human and of TBE-derivation by isoenzyme and chromosome analysis. The tumor tissue was subsequently transferred by xenograft inoculation of test animals with minced tumor tissue, and human tumor cell lines have been isolated from passages 7 and 9, respectively, of TBE-1 and TBE-1SA xenograft tumors. The xenograft tumors had shorter latency periods, and progressed to the ability to reach a 1 cm^2 sc tumor in 2 months after 4 to 6 passages in nude mice.

R9.8

ASSOCIATION OF $p60^{src}$ WITH TRITON-RESISTANT CELLULAR STRUCTURE CORRELATES WITH MORPHOLOGICAL TRANSFORMATION. M. Hamaguchi and H. Hanafusa. The Rockefeller University, New York, USA.

Association of $p60^{src}$ with a cellular structure resistant to non-ionic detergent extraction that consists primarily of cytoskeletal proteins was examined in cells infected with various mutants of RSV. Over 70% of wild type RSV $p60^{v-src}$ was associated with this structure. In contrast, both chicken and rat $p60^{c-src}$ were soluble to the detergent extraction. Furthermore, $p60^{c-src}$ was detergent-soluble even in transformed cells, suggesting that the association of $p60^{v-src}$ is not a result of cell transformation.

We found that nontransforming mutant $p60^{v-src}$, including non-myristylated forms that fail to associate with plasma membranes and those inactive in protein kinase, were essentially soluble by the detergent extraction. In contrast, the src proteins of transforming mutants derived from $p60^{c-src}$ by single amino acid substitutions were resistant to the detergent extraction. Results with these viruses and with additional RSV mutants showed a good correlation between the degree of association of $p60^{src}$ with the detergent insoluble structure and the extent of cell transformation. Thus, this interaction may play an important role in the process of transformation by RSV.

R9.9

ACTIVATED FORMS OF THE C-SRC GENE PRODUCT IN HUMAN RETINOBLASTOMA CELLS. John Bechberger+, Akio Tanaka++, and Donald J. Fujita++. Cancer Research Laboratory, U. of Western Ontario, London, Ont.+, and Dept. of Medical Biochemistry, U. of Calgary, Calgary, Alberta+

Retroviral oncogenes (v-src) represent altered forms of cellular proto-oncogenes (c-src), and have served to identify a wide variety of c-src gene products that may be altered or activated in cancers of humans and animals. The c-src protein appears to consist of at least five possible functional domains: membrane binding, recognition, modulatory, kinase, and regulatory. We have observed that there are two forms of the c-src protein differing by 1kd in apparent M.W. in a human retinoblastoma (Rb) cell line. The larger form appears to exhibit a higher protein tyrosine kinase (PKase) activity than the smaller one since the Rb cells exhibited PKase activity levels several fold higher than that of normal human fibroblasts. Analysis of the two c-src proteins revealed that the major difference in size resides within the amino-terminal 10-kdal region. Neither of these forms exhibited phosphorylation on Tyr residues within this amino-terminal region and phosphorylation was detected exclusively on Ser residues. Analysis of c-src m-RNA indicated that the Rb cells exhibited only one species of c-src mRNA having the same size as the transcript observed in normal fibroblasts. At least 3 possible explanations exist for the presence of two forms of c-src proteins: 1) Alternative RNA splicing mechanisms could create src proteins having differences within the putative recognition domain within the amino terminal region; 2) the larger form might be altered by modifications such as novel phosphorylations on Ser; 3) the larger form might be one of the other closely related src-type protein kinases which happens to cross react with the monoclonal v-src antibody used. In order to examine these possibilities further, antibodies specific for the c-src proteins are being made using human c-src proteins expressed to high levels in E. coli. These results will be discussed.

R9.10

ISOLATION OF NOVEL TYROSINE PROTEIN KINASES: USE OF ANTIPEPTIDE ANTIBODIES DIRECTED AGAINST THE INSULIN RECEPTOR TYROSINE KINASE DOMAIN.
Andrew Ziemiecki*, Robert R. Friis*, Rolf G. Muller*, Denis Scanlon+, Ludwig Institute for Cancer Research, *Bern Branch, 3010 Bern, Switzerland; +Melbourne Branch, 3050 Melbourne, Australia.

We have raised antiserum against peptides corresponding to regions of high homology shared among 10 different members of the tyrosine-specific protein kinase family.

Antibodies were raised by repeated immunization of rabbits with peptide coupled to BCG purified protein derivative (PPD). Western blot analysis of IM9 cells (over-expressing the insulin receptor), A431 cells (over-expressing the EGF receptor) and NIH 3T3 cells stably transfected with several retrovirally-transduced tyrosine protein kinases revealed several protein bands lighting up specifically with immune serum.

We have additionally screened some 25 human tumor cell lines for elevated membrane-associated tyrosine protein kinase activity. Under the experimental conditions employed, cell lines previously known to overexpress tyrosine protein kinases like A431 and IM9 exhibited 20-35% of the total phosphate on tyrosine. Certain other tumor lines exhibited values between 10-18% and we have chosen 5 of these cell lines as potential sources for isolation and cloning of new tyrosine protein kinases.

R9.11

INTERFERENCE OF ONCOGENE PRODUCTS WITH THE HORMONE DEPENDENT MMTV-LTR TRANSCRIPTION
R. Jaggi, R. Friis, A. Schläfli and B. Groner, Ludwig Institute for Cancer Research, Bern Branch, Inselspital, 3010 Bern

Several oncogene proteins repress the glucocorticoid-dependent transcription of the MMTV-LTR (EMBO J. 5, 2609-2616, 1986). To probe the mechanism of this effect NIH cells transfected with a MMTV-LTR gene construct or with a chimeric gene construct consisting of the hormone responsive element of the MMTV-LTR (HRE) and the a-globin gene were infected with ras-, mos-, src- or myc-expressing retroviruses. The kinetics of transcriptional induction and subsequent repression of the hormone-dependent gene constructs was determined. Stimulation with dexamethasone and treatment with cycloheximide did not change the pattern of induction and repression of the HRE-a-globin gene transcription in ras-, mos- and src-infected cells. We conclude that the hormone/receptor binding domain is instrumental for the repression of the MMTV-LTR transcription and suggest that the inhibition of the transcription is mediated either by a modification of the activated glucocorticoid receptor/MMTV-LTR DNA complex, or by other transcription factors binding to HRE of MMTV.

R9.12

A NEW "SENSE" RNA APPROACH TO BLOCK VIRAL RNA REPLICATION.

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The use of "antisense" RNA is being widely considered to block specific steps in viral infections. We have envisaged a new "sense" RNA approach to block viral RNA replication in vitro and possibly in vivo. In the turnip yellow mosaic virus (TYMV) system, the recognition site for the viral replicase (RNA-dependent RNA polymerase) is assumed to be located within the 3' end of the RNA genome. Small "sense" RNAs have been obtained by in vitro transcription of the corresponding cloned cDNAs. Replication of TYMV RNA in vitro was shown to be blocked only by those small "sense" RNAs that contain the 3' terminal region of the genome. This "sense" RNA approach could be of general potential use in antiviral research.

R9.13

tRNA-LIKE REGIONS IN PLANT VIRAL RNAs: STRUCTURE, RECOGNITION BY SPECIFIC PROTEINS, FUNCTIONAL ASPECTS.

Rajiv L. Joshi, Anne-Lise Haenni.

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The 3' regions of many plant viral RNA genomes can interact with certain tRNA-specific proteins and thus display tRNA-like recognitory properties.

The structural features of the tRNA-like regions in the viral RNAs underlying recognition by tRNA-specific proteins have been intriguing us for many years and recent studies have indeed led to particularly exciting results. The tRNA-like regions in the valine-, tyrosine- and histidine-accepting viral RNAs adopt conformations reminiscent of the 'L'-shape of tRNAs in completely different manners.

Because of these peculiar foldings, these viral RNAs have been used as model substrates to elucidate the RNA conformational requirements of certain tRNA-specific proteins.

The biological function of the interaction between these plant viral RNAs and tRNA-specific proteins still remains most puzzling at the present time. The different hypotheses that have been proposed, essentially centered around translation or replication, will be discussed in light of all the data available to date.

R10.1

MECHANISM OF ANTIBODY DEPENDENT ENHANCED VIRULENCE OF YELLOW FEVER VIRUS. E A Gould & A Buckley. Arbovirus Research Unit. London School of Hygiene and Tropical Medicine, United Kingdom.

Some monoclonal antibodies against yellow fever (YF) virus envelope protein can enhance the neurovirulence of YF virus for mice when administered passively. Antibody dependent enhancement (ADE) can only be demonstrated with strains that reach high titres in the brains of immunologically mature mice. The precise conditions under which the experiments are carried out are critical to the demonstration of the phenomenon of enhancement.

The underlying mechanisms of ADE have been investigated in detail. On the basis of biological, immunological and pathological data, enhancement appears to be mediated through accelerated inflammatory responses resulting from antigen/antibody interactions on the surfaces of infected brain cells.

R10.2

HETEROLOGOUS FLAVIVIRUS INFECTION-ENHANCING ANTIBODIES IN SERA OF NIGERIANS.

A. H. Fagbami[1]; Halstead S. B.[2]; Marchette N.[2] and Kay Larsen[2].

[1]Department of Virology, College of Medicine, University of Ibadan, Ibadan, Nigeria and [2]Department of Tropical and Medical Microbiology, University of Hawaii, Honolulu, Hawaii 96816.

Human sera collected from Nigeria were examined for plaque reduction neutralising and infection-enhancing antibodies against Dengue-2 (DEN-2), Yellow Fever (YF), and West Nile (WN) viruses.
Neutralisation tests showed that 17 of 19 sera tested contained flavivirus antibody; 11 were positive to all 3 viruses, 5 to dengue and Yellow fever, 1 to dengue virus only, and two had no detectable antibodies to any of the flaviviruses tested. Enhancement assays showed that 17 flavivirus N positive sera contained infection-enhancing antibodies to DEN-2, and 16 had to YF. Although 11 sera were positive for WN N antibody, 17 enhanced West Nile virus. Heterologous infection-enhancing antibodies titres were lower than the homologous. Broadly reacting sera and those with high N antibody titres produced the highest infection-enhancing antibody titres.

R10.3

NOVEL POLYPROTEINS INDUCED IN FLAVIVIRUS AND ALPHAVIRUS-INFECTED CELLS BY HYDROXYLEUCINE.

G.R. Crawford, G.W. Smith, P.J. Wright. Department of Microbiology, Monash University, Clayton, Victoria, Australia.

The effects of the leucine analogue threo-β-hydroxy-DL-leucine (THL) on protein synthesis in infected Vero cells were investigated. Cells were infected with the flavivirus Kunjin (KUN) or dengue virus type 2 (DEN-2), or with the alphavirus Semliki Forest virus (SF), and radiolabelled in the presence of THL. Novel proteins larger than NS5 (NV5 or P98) were detected in KUN and in DEN-2 virus-infected cells. The major DEN-2 proteins were gp220, gp165, gp140, gp128, gp120 and gp89; each of these polypeptides bound to concanavalin A. In cells infected by SF virus, eight novel proteins larger than E1 were detected, the largest of M_r 145,000; none of these proteins bound to concanavalin A or were radiolabelled with [^{3}H]mannose. The results are consistent with the proposal that the incorporation of THL into viral proteins may inhibit (i) the insertion of the proteins into membranes because of changes in the hydrophobicities of signal peptides and (ii) the proteolysis of precursor polyproteins.

R10.4

IDENTIFICATION OF CIS-ACTING SEQUENCES ESSENTIAL FOR THE REPLICATION OF SINDBIS VIRUS RNAS. R. Levis, P. White, A. Grakoui, C. Rice, S. Schlesinger and H. Huang. Department of Microbiology and Immunology, Washington University School of Medicine, St. Louis, MO. USA.

We constructed cDNA clones containing either the full-length Sindbis genome or a Sindbis defective interfering (DI) RNA and inserted them into a plasmid containing the SP6 bacteriophage polymerase promoter. When transfected into chicken embryo fibroblasts, the transcribed full-length RNA produces infectious virions and the transcribed DI RNA is amplified in the presence of Sindbis helper virus. By making a series of deletions in the DI cDNA clone, we identified sequences at the 5' and 3' termini of the DI genome which are essential for DI replication and encapsidation. Non-Sindbis sequences, specifically the bacterial chloramphenicol acetyltransferase (CAT) gene, can be inserted into the deleted regions of the DI. After transfection, RNA transcribed from these clones is amplified and translated to produce CAT protein. We are now constructing a series of Sindbis DI clones which contain the junction region for subgenomic mRNA synthesis inserted upstream from the CAT coding sequence. By manipulating junction containing DI RNAs and assaying for subgenomic RNA synthesis in transfected cells, we hope to elucidate those sequences which are responsible for directing the synthesis of subgenomic RNA.

R10.5

CYTOTOXIC T CELLS AGAINST DENGUE-INFECTED TARGET CELLS. T.Pang, S.Devi, S.K.Lam, Department of Medical Microbiology, Faculty of Medicine, University of Malaya, 59100 Kuala Lumpur, Malaysia.

A cytotoxic T lympholyte (CTL) response to dengue virus-infected target cells is described. Effector cells were generated in an in vitro secondary culture and appeared to be T cells possessing both the Lyt 1.1 and Lyt 2.1 surface antigens. A stronger CTL response was noted with the H-2k haplotype compared to H-2d and H-2 compatibility was required between CTL and target cells. CTL generated showed some cross-reactivity with target cells infected with Japanese encephalitis virus (JEV), another flavivirus, but not with target cells infected with an alphavirus, Sindbis.

We believe this to be the first conclusive demonstration of a CTL response to dengue virus and the significance and importance of these findings will be discussed.

R10.6

Partial purification of West Nile virus (WNV) polymerase activity. J.Grun and M. Brinton, The Wistar Institute, Philadelphia, U.S.A. WNV polymerase activity in cytoplasmic extracts from virus-infected BHK-21 cells was concentrated by high speed centrifugation. The resulting microsomal pellet was free of nuclei, but contained ribosomes, mitochondria, endoplasmic reticular fragments, cellular transferases, which competed with the viral polymerase activity for radiolabeled UTP and ATP, and considerable amounts of Ca^{++}, which were inhibitory to viral polymerase activity. In order to separate WNV polymerase activity from these components and other inhibitory cellular factors contained in microsomal pellets, and to facilitate studies of the protein and RNA composition of flavivirus replication complexes, we have affected a further purification of polymerase activity. Utilizing nondenaturing discontinuous glycerol gradients, continuous sucrose gradients and sepharose 2B column chromatography, polymerase activity was successfully separated from numerous cell proteins as well as from the largest nonstructural viral protein, NS5. Since WNV replication complexes remained tightly associated with membrane fragments further purification of active complexes will require the use of membrane solubilizing detergents. As a prerequisite to such studies, the polymerase activity contained in partially purified fractions was evaluated for its sensitivity to NP_{40}, lubrol-PX, deoxycholate and tween 20. Only in the presence of tween 20 was 100% of the polymerase activity retained as compared to nondetergent treated controls.

R10.7

THE SMALL NONSTRUCTURAL PROTEINS OF FLAVIVIRUSES: POSITIVE IDENTIFICATIONS AND PRECISE GENE LOCATIONS. E.G. Westaway, G. Coia, M.D. Parker and G. Speight. Department of Microbiology, Monash University, Melbourne, Australia.

The deduced amino acid sequence of the 10,302 nucleotides in the coding region of the Kunjin virus genome was compared with partial N-terminal amino acid analyses of all the nonstructural (ns) proteins. It was thus possible to positively identify the exact locations and the products of the previously hypothetical flavivirus genes NS2A, NS2B and NS4B. The hypothetical ns4a gene product was not identified. The cleavage sites in the polyprotein of the established NS2A and NS4B products did not involve double basic amino acids as originally proposed for most ns proteins of yellow fever virus, as a working hypothesis. Putative cleavage sites homologous to those established for Kunjin virus were present in all published flavivirus amino acid sequences, indicative of conservation of the sites.

The proteins translated from the NS2A, NS2B and NS4B genes were apparently rapidly truncated after synthesis in the carboxy-terminal region because their molecular weights as measured by gel electrophoresis were smaller by 5,000 to 7,000 than the coding content of their genes. The fate of these lost polypeptides and the mechanism of their degradation are unknown.

R10.8

THE SYNTHESIS AND MODIFICATION OF IMMUNOGENIC POLYPEPTIDES ENCODED BY TICK-BORNE ENCEPHALITIS VIRUS. J R Stephenson, A J Crooks and J M Lee, VBPL, CAMR, PORTON DOWN, SALISBURY, WILTS.

Tick-borne encephalitis virus (TBEV) encodes three structural proteins E, C & M, Sera from infected animals, however, contains antibodies to only one virion protein, E, but in addition contains antibodies to the non-structural protein NV3. Both the E and NV3 polypeptides contain galactose, glucosamine, fucose and mannose and most of these sugars are N-linked. NV3 is present in an extracellular form which appears to be more heavily glycosylated than its intracellular counterpart. NV2, the putative precursor to M, is also glycosylated and is never seen in purified virus particles. Sequence data from other flaviviruses suggest that a large polypeptide precursor is made from which the mature proteins are cleaved. Kinetic studies of the synthesis of the E and NV3 polypeptides revealed no similar precursor for TBEV proteins, but indicated that the synthesis of the two major immunogens is dependant upon the translocation of the ribosome complex to subcellular compartments which contain enzymes capable of cleaving the nascent polypeptide chain.

R10.9

THE MOLECULAR EPIDEMIOLOGY OF DENGUE VIRUS.
D.W. Trent, J.A. Grant, A.V. Vorndam, G. Fox and T.P. Monath Div. of Vector-Borne Viral Diseases, Centers for Disease Control, Ft. Collins, Colorado U.S.A.

Dengue (DEN) is a world-wide health problem with epidemics involving thousands of persons recurring in areas of tropical Asia, Africa, and the Americas where Ae. aegypti mosquitoes are present. Genetic variation in DEN-1, 2, and 3 viruses from different geographic areas has been examined by computerized analysis of 40S genomic RNA oligonucleotide fingerprints (OnFp). Direct comparison of OnFp revealed that viruses of the same serogroup may be genetically distinct and that isolates during epidemics within a region are genetically similar. African Ae. aegypti and forest mosquito transmitted DEN-2 are distinct; Ae. aegypti transmitted viruses cause most human infections. DEN-2 viruses from India, the South Pacific and the Caribbean are similar, suggesting that pandemics in these regions have resulted from the introduction of new virus strains. Two genetically distinct DEN-2 viruses are being transmitted to humans in the Americas. Asian, African, South Pacific, and New World DEN-1 isolates are genetically distinct as are DEN-3 strains from Thailand and Indonesia. We have identified 14 genetic variants of DEN-2, 5 of DEN-1, and 2 of DEN-3. Genome sequence analysis, genotype specific probes, and computerized analysis of OnFp have greatly improved our understanding of DEN virus epidemiology and genetic variation.

R10.10

THE ANTIGENIC RELATIONSHIP OF WEST NILE STRAINS.
N. Blackburn, T.G. Besselaar. Arbovirus Unit, National Institute for Virology, Johannesburg.

The antigenic relationship of several South African West Nile (WN) isolates, the South African WN prototype strain H442, the Egyptian strain EG101 and the Indian strain G2266 were compared using two methods. The first was by titre ratios or 'R' values calculated from homologous and heterologous neutralisation titres. The second was by testing monoclonal antibodies (MAb) against the various WN strains using the indirect immunofluorescent test.

The titre ratios demonstrated major differences between the WN strain H442 and the local isolates, and these viruses against EG101 and G2266. The qualitative results from the study using MAb's, showed that at least 15 of the 17 MAb's prepared against the strain H442 recognized epitopes on most of the isolates and on strain EG101. Six of the panel of 17 MAb's did not react against one of the isolates, and a similar number against strain G2266. These viruses were therefore considered to be significantly different to strain H442. The quantitative results, the importance of which still has to be established, were similar to those obtained using titre ratios.

R10.11

NEUROVIRULENCE IN THE MURINE ENCEPHALOMYELITIS CAUSED BY SINDBIS VIRUS. A.C. Jackson, T.R. Moench, B.D. Trapp, and D.E. Griffin. Departments of Neurology and Medicine, The Johns Hopkins University, Baltimore, Maryland, USA

Neuroadapted Sindbis virus (NSV) has been selected by serial passage of wild-type Sindbis virus (SV) in mouse brain. Following intracerebral inoculation, NSV causes a severe encephalomyelitis with hindlimb paralysis and high mortality; SV causes mild disease that is non-fatal. Structural differences in SV and NSV have been identified in the envelope glycoproteins. In order to determine the biologic basis of neurovirulence, these viral infections have been compared using infectivity assays, light and electron microscopy, in situ hybridization, and immunohistochemistry.

More infectious virus is present in the central nervous system (CNS) during NSV infection than during SV. Both viruses enter the CNS via the ependyma and spread to gray matter areas, including the anterior horns of the spinal cord. Their cellular targets are not different, and neuronal infection is prominent. NSV infects more neurons and causes more severe injury than SV. Marked swelling of dendrites and axons is observed by light microscopy in thoracic and lumbar neurons in only NSV infection. Neuroadaptation likely occurs by increasing the efficiency of viral replication in neurons, rather than by a fundamental change in the CNS cellular tropism or the topography of the infection.

R10.12

GENETIC STUDIES OF A VIRULENT DENGUE VIRUS AND ITS ATTENUATED CANDIDATE VACCINE VIRUS
J. Blok, H. Butler, B. Herring, S. McWilliam and S. Yoksan*
Queensland Institute of Medical Research, Brisbane, Australia and *Dept of Pathology, Ramathibodi Hosp., Mahidol Univ., Bangkok, Thailand

Dengue haemorrhagic fever is a major paediatric health problem in South-east Asia. A dengue-2 virus isolate from a fatal case in Thailand was attenuated through serial passage in primary dog kidney cells in an attempt to make a candidate dengue vaccine. The parental and attenuated viruses were then grown in mosquito cells (C6/36) to produce large amounts of virus for genetic analysis.

The single stranded viral RNA (about 11Kb) from these viruses are being compared by (1) restriction enzyme mapping of cDNA and dsDNA and (2) cloning and sequencing the complete genome. Thirteen restriction enzymes were used in the mapping study and these maps represent about 15% of the genome. These studies revealed that there was ca. 3% difference between the parental and attenuated virus strains. To date, nucleotide sequence data from both strains extend over 40% of the genome and reveal that there are 12 base changes, 3 of which are silent changes. The sequences represent portions of the structural proteins C, pre M and E, and non-structural proteins NS1, ns2a, ns2b, NS3, ns4a, NS5, and all of the non-structural protein ns4b.

R10.13

CLONING AND SEQUENCING OF TBE VIRUS cDNA AND ANALYSIS OF ANTIGENIC VARIANTS

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The genome of Tick Borne Encephalitis (TBE) virus - like those of other flaviviruses - consists of a single-stranded RNA molecule of approximately 10500 nucleotides length that also serves as a messenger, but lacks a polyA structure at its 3'-end.

Both random sequence and TBE-specific oligonucleotide primers were employed for the synthesis of cDNA. After cloning into bacterial plasmids, cDNA inserts ranging in size from 2000-4000 bp were identified and used for sequence analysis. Sequence information of different TBE virus isolates was consequently obtained by directly sequencing the RNA genomes. Sequence data and a comparative computer analysis with other known flavivirus sequences are presented.

Moreover, variants of TBE virus were selected by growing the prototype strain (Neudoerfl) in cell culture in the presence of neutralizing monoclonal antibodies. Escape mutants are used to identify primary sequences of antigenic epitopes localized on the viral envelope protein. Results of the analysis of several variants of TBE virus are presented.

R10.14

DETERMINANT OF VIRULENCE IN SEMLIKI FOREST VIRUS ENCEPHALITIS. J.K. FAZAKERLEY, S. Mehta, S. Pathak, H.E. Webb. Neurovirology Unit, St Thomas' Hospital, London, UK.

The susceptibility of the mouse to the encephalitic alphavirus, Semliki Forest virus, varies with the age of the mice and the strain of the virus. The L10 strain of SFV is virulent for mice of all ages. The A7(74) strain is virulent for neonatal mice, but decreases in virulence with increasing age of the mice. Following infection of neonatal and baby mice, both viruses replicate to high titres in the CNS, resulting in death of the mice within 4 days. The L10 strain behaves identically in adult mice. Infection of mice >21 days with the A7(74) strain is not fatal, and virus is detectable in the brain, by infectivity for 12 days and by immunocytochemistry for months. As with the L10 strain, EM studies indicate that A7(74) infects predominantly neurones, but in contrast to L10, no mature virus or advanced stages of viral replication are seen, even when the A7(74) brain titres are as high as, or higher than the titres in moribund L10 infected mice. Results suggest that the determinant of virulence for the A7(74) strain is the interaction of the virus with age specific host cell membranes.

R10.15

CHARACTERIZATION IN VITRO OF SINDBIS VIRUS ts6.

D.L. Sawicki, D.J. Barton and S.G. Sawicki, Department of Microbiology, Medical College of Ohio, Toledo, Ohio, 43699 U.S.A.

We established an in vitro Sindbis virus (SV) transcription system using the membrane fraction (P15) of infected baby hamster kidney cells. Alpha^{32}P-labeled CTP was incorporated into RNA that migrated on formaldehyde agarose gels as replicative-intermediate (RIs), 49s genomic and 26s subgenomic RNA and could be chased from RIs to 49s and 26s RNA. Incorporation proceeded linearly for 5 to 30 minutes depending on reaction conditions and stopped when CTP was degraded to CDP, CMP and inorganic phosphate. Our in vitro transcription system did not appear to initiate RNA synthesis, but only elongated RNA chains that had been initiated in vivo. Using this in vitro transcription system, we determined if SV(ts6) of the F complementation group of HR ts mutants had a ts mutation affecting the initiation or the elongation of viral RNA.

R10.16

EXPRESSION OF JAPANESE ENCEPHALITIS VIRUS ENVELOPE GLYCOPROTEIN E GENE IN EUKARYOTIC CELLS. H. Fujita, S. Manabe, C.Mori, M. Takagi, I. Yoshida, K. Fukai The Research Foundation for Microbial Diseases of Osaka Univ., Osaka, Japan. H. Sumiyoshi, K. Morita, I. Fuke, A. Igarashi Department of Virology. Institute for Tropical Medicine. Nagasaki Univ., Nagasaki, Japan.

The Japanese encephalitis (JE) vaccine has been produced from JEV-infected mouse brain. But that vaccine is limited in its amount for wide-scale immunization in epidemic areas in the East to South Asia. In order to develop the second generation vaccine, we tried to express JEV envelope glycoprotein E gene in yeast.

cDNA clone covering E gene was connected to yeast repressible acid phosphatase gene promoter and introduced to transform S. cerevisiae. The E gene expression was induced in phosphate-free synthetic medium. The 53 kd protein was identified as E gene product by Western blotting analysis and purified with ion-exchanger, isopicnic banding in CsCl or affinity column chromatography. Immunization of mice with yeast-derived JE E protein produced limited but definite levels of anti JE antibodies which could neutralized JE virus. Now we are making another expression plasmid for mammalian cells. The results could be regarded as encouraging for future technical improvement toward the practical second generation JE vaccine.

R10.17

INTERFERON INDUCTION FROM NON-IMMUNE LYMPHOCYTES BY AUTOLOGOUS DENGUE VIRUS-INFECTED MONOCYTES. I.Kurane and F.A.Ennis, Department of Medicine, University of Massachusetts Medical School, Worcester, MA. USA.

We have analyzed the interaction between the peripheral blood lymphocytes (PBL) of non-immune donors and dengue virus-infected cells, which results in interferon (IFN) production. PBL produced IFN when cultured with dengue virus-infected autologous monocytes. Dengue virus-infected monocytes treated with glutaraldehyde, which produce no infectious dengue virus, also induced IFN. The ability of dengue-infected cells to induce IFN correlated with the appearance of dengue antigens in the infected cells. Characterization of IFN-producing PBL by FACS-sorting using monoclonal antibodies demonstrated that the predominant IFN-producing cells were contained in the HLA-DR+ and T3- subsets. The IFN produced contained high titers of IFNα (150-1000IU) and a low level of IFNγ detected by radioimmunoassay employing monoclonal antibodies to IFNα and IFNγ. Treatment of monocytes with 400IU of IFNα before dengue virus infection decreased the yield of infectious virus more than 99%, and the percentage of dengue-antigen positive monocytes by 90%. These results suggest that IFN produced by PBL in response to dengue virus-infected monocytes may have an important role in controlling dengue virus infection, and aid in avoiding the severe complications of dengue infection; hemorrhagic fever and shock.

R10.18

EVIDENCE FOR A DIMER FORM OF THE FLAVIVIRUS SOLUBLE COMPLEMENT FIXING ANTIGEN (NS1). G. Winkler, T. Ryan, V. Randolph, G. Cleaves and V. Stollar. Dept. of Molec. Genetics and Microbio., UMDNJ-R.W. Johnson Med. Sch., Piscataway, NJ, U.S.A. 08854

The nonstructural flavivirus glycoprotein NS1 has properties which are unusual for a nonstructural protein, including the ability to induce protective antibodies. When we analyzed lysates of 35-S-met-labeled dengue-2 infected mosquito cells which had not been boiled prior to PAGE we found very little NS1 (mol.wt. 46000). Instead a new glycoprotein was present which had a mol.wt. of 86000 (gp86). When the lysate was boiled for several minutes gp86 disappeared and NS1 appeared. This conversion of gp86 to NS1 was effected also by low pH (<3), but not by SDS, 2-mercaptoethanol, guanidine/HCl or high salt. That NS1 was actually present in the gp86 band was shown by Western blotting and immunoassay using a monoclonal antibody to dengue-2 NS1. Using the same technique we were also able to demonstrate gp86 in the brains of dengue-2 infected suckling mice. High mol. wt. forms of NS1 were also found in unboiled lysates of BHK cells infected with dengue-2, St. Louis enceph. or Powassan viruses as well as in the medium of infected cells. In each case boiling caused conversion to NS1. Pulse chase experiments with 35-S methionine suggested that NS1 did not accumulate but was largely converted to gp86 within 40 min. Determination of the glycosylation pattern with endo F and H indicated that gp86 is a dimer of NS1. Our results are consistent with the idea that NS1 exists as dimer in infected cells and that this may be the functional form.

R10.19

MOUSE MONOCLONAL ANTIBODIES TO ARBOVIRUSES. C. Heuland, O. EL-Badry and S.S. VedBrat. Braton Biotech, Inc. 1 Taft Court, Rockville, Md. 20850

Hybridoma cells secreting monoclonal antibodies (Moabs) were generated to three members of the Arbovirus family, two Bunyaviruses, Chagres and Sandfly-Naples, and one Flavivirus, West Nile. Moabs to Chagres virus were produced by immunizations with partially inactivated virus, and those to Sandfly-Naples with live virus. Hybridomas were subsequently selected by an Immunofluorescence assay (IFA) using virus infected Vero cells as target, and uninfected Vero cells as the negative control. Moabs to West Nile virus were generated and selected by enzyme linked immunosorbent assay (ELISA) using inactivated West Nile virus obtained as mouse brain homogenates. Sandfly-Naples nucleocapsid protein in mouse brain homogenate was used as a negative control. None of the Moabs showed cross reactivity to any of the other related viral agents tested. Isotype analysis by IFA and ELISA revealed all three Chagres virus Moabs to be of the IgG1K type; three of the Sandfly-Naples virus Moabs to be of the IgG2aK class and one IgMK; and the West Nile virus Moabs to be one each of the IgG2aK, IgG2bK and IgG1K classes. This work was supported by USAMRDC contract # DAMD17-85-C-5196.

R10.20

MOLECULAR CLONING OF DEN-1 STRUCTURAL GENES AND ANALYSIS OF GENETIC DIVERGENCE AMONG DEN-1, 2 AND 4. M.C. Chu and D.W. Trent. Division of Vector Borne Viral Diseases, Centers for Disease Control, Ft. Collins, Colorado, USA.

Conserved and unique nucleotide regions coding for dengue (DEN) envelope epitopes that are involved in virus neutralization and antibody-dependent enhancement (ADE) have yet to be defined. The nucleotide sequence encoding the structural proteins of a Thailand DEN-1 human isolate has been determined. Two overlapping cDNA clone of 2,512 nucleotides contained a 5'non-coding region of 128 nucleotides, followed by a single open reading frame that coded for the structural proteins and the amino terminus of the NS1 nonstructural protein. The order of the DEN-1 structural proteins (5'-capsid-preMembrane-membrane-envelope-NS1-3') is identical to that reported for other flaviviruses. Comparison of the deduced amino acid sequence of the entire structural region of DEN-1 with the published sequences of other serotypes reveals that it shares 63% overall homology both with DEN-2 (Jamaica) and DEN-4 (Dominica). DEN-2 and DEN-4 share 55% homology in the same region. The percentages of identical amino acids between individual proteins of DEN-1 and either DEN-2 or DEN-4 were: capsid, 61% and 60%; pre-membrane/membrane, 61% and 73%; and envelope, 65% and 60%; respectively. Detailed examination of DEN sequence homologies in the envelope protein has permitted identification of epitopes that may mediate neutralization and ADE.

R10.21

SYNTHETIC PEPTIDES DERIVED FROM THE GENE SEQUENCE OF THE E2 GLYCOPROTEIN OF VENEZUELAN EQUINE ENCEPHALITIS VIRUS DEFINE AN IMMUNODOMINANT DOMAIN. J. Roehrig and A. Hunt. Division of Vector-Borne Viral Diseases, U. S. Centers for Disease Control, Ft. Collins, Colorado, U.S.A.

We have previously defined eight epitopes on the E2 surface glycoprotein of Venezuelan equine encephalitis virus (VEEV), strain TC-83, using murine monoclonal antibodies (MAbs). Subsequent gene sequencing of TC-83 has allowed us to build synthetic peptides corresponding to the E2 protein using Merrifield solid phase synthesis. We are using these peptides to identify the E2 epitopes.

An overlapping set of peptides, 25 amino acids long, and representing the N-terminal one-half of this protein have been synthesized and analyzed. A VEEV, TC-83 specific site has been identified on the N-terminus. Polyclonal virus antibody defines an immunodominant domain from amino acid 120 to 225. Sequence analysis of MAb derived neutralization escape variants corroborates this observation, with most MAb defined epitopes located within this domain. Immunization of mice with unconjugated peptides indicates that peptides encoding this domain are the most efficient at eliciting antibody capable of reacting with virus. The murine anti-virus response appears to be genetically restricted with different haplotypes of mice reacting to different peptides.

R10.22

DEPENDENCE ON THE BIRTH SEASON OF THE LEVELS OF ANTIBODIES AGAINST JAPANESE ENCEPHALITIS, WEST NILE AND DENGUE VIRUSES IN THE PAKISTANI POPULATION. M. Sugamata, T. Miura Department of Hygiene, Teikyo University School of Medicine, Itabashi, Tokyo 173, JAPAN.

It has been known that among Japanese people, those born in the summer (Japanese encephalitis(JE) epidemic season) of JE epidemic years showed a higher morbidity of JE, a lower efficacy of JE vaccination, and a lower HI antibody positive rate than those born in the other months of the same epidemic years. These phenomena imply that those born in a JE epidemic season of epidemic years may have been latently infected with JE virus at a certain pre- or perinatal stage of development of their immune systems, thus acquiring a different responsiveness to JE virus infection in later years. For the purpose to ascertain the validity of this hypothesis by finding an evidence plausible for it in other countries, 151 paired serum samples were collected from healthy Pakistani persons living in Karachi, Pakistan, in July and October, 1985.

Significantly lower levels and lower responsiveness of antibodies were observed against the three flaviviruses (JE, West Nile and dengue) among healthy Pakistani persons born during the period between February and June than among the others. No differences were detected against the other viruses (measles, rubella, herpes simplex-1 and Entero-70) by the season of their birth.

R10.23

PRODUCTION AND CHARACTERIZATION OF ANTISERA TO YELLOW FEVER FUSION PROTEINS PRODUCED IN E. COLI. T.J. Chambers, C. M. Rice Dept. of Microbiol. and Immunol., Washington Univ. Sch. of Med., St. Louis, MO., USA.

Specific subregions of a yellow fever virus 17D genome cDNA library have been subcloned into plasmid expression vectors under the control of the tryptophan operon. Trp E fusion proteins corresponding to the viral structural proteins prM and E, and the nonstructural proteins NS1, NS3, NS5, and subregions ns2a, ns2b, ns4a, and ns4b have been generated and used as immunogens. Production of a stable capsid fusion protein is still in progress.

Polyclonal rabbit antisera to these proteins are being characterized. Monospecific sera recognizing the viral nonstructural proteins NS3 and NS5 have been generated. In addition, antisera to the hydrophobic subregions ns2b and ns4b immunoprecipitate proteins of approximate MW 10,000 and 30,000, respectively. Characterization of the hydrophobic nonstructural gene products and investigation of viral polyprotein processing are underway.

R10.24

FINE MAPPING OF SINDBIS RNA(-) ts MUTANTS: CAUSAL LESIONS RESPONSIBLE FOR COMPLEMENTATION GROUP F MUTANTS. Y.S. Hahn[1], A. Grakoui[2], J. H. Strauss[1], C. M. Rice[2]. [1]Division of Biology, Caltech, Pasadena CA, and [2]Dept. of Microbiol. and Immunol., Washington Univ. Sch. of Med., St. Louis MO, USA.

Complementation group F mutants ts 6, ts 110, and ts 118 are defective in RNA synthesis at the nonpermissive temperature. The most well characterized member, ts 6, ceases genomic, subgenomic, and (-) strand RNA synthesis upon shift to nonpermissive conditions. cDNA clones of this mutant as well as temperature insensitive revertants have been constructed. To assign this phenotype to a specific locus in the viral genome, restriction fragments from the mutant cDNA clones were used to replace the corresponding regions of a full-length cDNA clone of Sindbis. These plasmids were transcribed in vitro by SP6 RNA polymerase to produce infectious transcripts which were then assayed for production of ts virus. The causal mutation for the ts 6 phenotype was shown by this method to map between nucleotides 5284 and 6267, and sequence analysis indicates that a single base substitution in nsP4 resulting in a replacement of glycine by glutamate at position 152 (from the N-terminus) is responsible for the ts 6 defect. ts 110 and ts 118 do not contain mutations in this portion of nsP4 and experiments are underway to localize their ts lesions. It is of interest that the ts 6 defect is N-terminal to the region of nsP4 conserved with the poliovirus RNA-dependent RNA polymerase, and it remains to be established whether this defect directly effects polymerase activity or inhibits some other common step in the synthesis of all viral RNA species.

R10.25

SINDBIS VIRUS MUTANTS RESISTANT TO METHIONINE DEPRIVATION HAVE AN ALTERED METHYLTRANSFERASE. L.M. Scheidel, R.K. Durbin, V. Stollar. Dept. of Molecular Genetics and Microbiology, UMDNJ-R.W. Johnson Medical School, Piscataway, NJ, U.S.A. 08854

Sindbis virus (SVstd) replication in Aedes albopictus mosquito cells is sensitive to methionine deprivation. Evidence has been obtained indicating that this sensitivity is due primarily to a decreased pool of S-adenosylmethionine (ado met) and the resulting failure to methylate the mRNA cap. Serial passage of SVstd on mosquito cells maintained in low concentrations of methionine allowed us to select mutants of SVstd (SVlm) whose replication was resistant to methionine starvation. Our hypothesis was that the resistance phenotype reflected an alteration in the viral methyltransferase increasing the affinity for ado met. We now report the use of an in vitro assay for the viral methyltransferase--using lysates from infected cells and guanine nucleotides as methyl acceptors--which kinetically distinguishes the SVstd and SVlm enzymes. This distinction was apparent only at low concentration (6.25 µM) of ado met. At a higher concentration of ado met (100µM) the rates of product formation catalyzed by the SVstd and SVlm enzymes were similar. Estimates of the Km for ado met of these enzymes are being determined.

R10.26

CELL SURFACE EXPRESSION OF THE YELLOW FEVER VIRUS (YF) NONSTRUCTURAL GLYCOPROTEIN NS1. J.J. Schlesinger, M.W. Brandriss, E.E. Walsh. Department of Medicine, Rochester General Hospital, University of Rochester, Rochester, New York, USA.

Immune recognition of viral antigen on the surface of infected cells may play an important role in the host defense against some viral infections. We used monoclonal (MAB) and monospecific polyclonal antibodies against the YF envelope protein (E) and YF nonstructural glycoprotein, NS1, as probes in an effort to detect plasma membrane expression of these antigens in infected neuroblastoma cells (Neuro 2a). Complement (C')-dependent cytolysis of YF-infected Neuro 2a was mediated by 9 of 11 IgG2a/2b anti-NS1 MAB and by monospecific rabbit anti-YF NS1 or anti-dengue NS1 antibody. However, none of 12 IgG2a/2b anti-E MAB or monospecific rabbit antibody to YF or dengue E mediated lysis. Using monolayers of viable infected Neuro 2a at 4°C, binding of radioiodinated anti-NS1 but not anti-E MAB was demonstrable. Productive infection was reduced up to 1000-fold in synchronously infected Neuro 2a maintained in medium containing IgG_{2a} anti-NS1 MAB in the presence but not absence of C'. These results may provide insight into the mechanism of recently described (J Virol 60:1153, 1986) protective immunization with NS1.

R10.27

CELL FUSION IN FLAVIVIRUS INFECTED MOSQUITO CELL CULTURES INDUCED BY LOW PH. V. Randolph, V. Stollar. Dept. of Molecular Genetics and Microbiology, UMDNJ-Robert Wood Johnson Med. Sch., Piscataway, NJ, U.S.A. 08854-5635

Cell-to-cell fusion triggered by exposure to low pH has been observed in both vertebrate and mosquito cell cultures infected with alphaviruses. In contrast, low-pH-induced fusion has not been reported in flavivirus infected vertebrate or invertebrate cells, although syncytia formation is frequently seen under normal culture conditions in some flavivirus infected mosquito cells. We report here that cell-to-cell fusion of a cloned line of Aedes albopictus cells infected with dengue and St. Louis encephalitis (SLE) flaviviruses was induced by exposure to low pH. Fusion was maximal in cultures 36 to 48 hrs post-infection and occurred when cultures were maintained at the acid pH for 15 min at 35°C. Fusion was more extensive in cultures infected with dengue virus than in cultures infected with SLE virus. The optimal pH range for fusion was 5.0 to 6.5 for dengue virus infected cells and 5.0 to 5.5 for SLE virus infected cells. Fusion was not observed in vertebrate cells (Vero and BHK) under these conditions despite similar virus yields. Fusion could be prevented by the addition of polyclonal antiviral antibodies and monoclonal antibody to the envelope glycoprotein, but was unaffected by the addition of polyclonal antibody to the NS1 nonstructural protein (which is also expressed on the cell surface).

R10.28

MOLECULAR BIOLOGY OF DENGUE VIRUS NS-1 PROTEIN. R. Putnak, P. Charles, R. Padmanabhan, C. Hoke and D. Burke. Department of Virus Diseases, Walter Reed Army Institute of Research, Washington, D.C., U.S.A.

NS-1 is a nonstructural glycoprotein. In dengue virus infected cells we have found several forms, differing in MW, in rough and smooth E.R., at the cell surface and secreted.

To begin to study its structure and function we have cloned it from dengue 2 virus, sequenced and expressed it. Comparison with Den 1 and 4 NS-1 showed that ca. 60% of the a.a. residues were conserved among all three serotypes, 70% between any two serotypes and 80% matched those of either Den 1 or 4. The longest uniformly conserved stretch was 13 a.a.; several stretches of 9-12 conserved a.a. were found. There is less conservation in the vicinity of the two glycosylation sites although the signals themselves are conserved. The poorest conservation was found within the C-terminal 60 a.a., especially in a 20 a.a. hydrophobic tail. Den 2, like other flaviviruses, was found to have a hydrophobic signal-like sequence preceeding the N-terminus of NS-1.

NS-1 was expressed in E. coli as a B-gal fusion protein from an M13 vector. Two proteins, differing in MW, reacted with NS-1 antibody. Processing of NS-1 was not affected by removal of B-gal from its C-terminus. We have also used M13 to express sub-genomic regions of NS-1. The N-terminal signal sequence appeared to be required for expression or stability of NS-1 protein. This sequence may be processed in E. coli.

R10.29

EPIDEMIOLOGY OF JAPANESE ENCEPHALITIS IN WEST BENGAL STATE, INDIA, WITH SPECIAL REFERENCE TO ITS VECTOR. A. Hati, S. Bhattacharya, D. Saha. Department of Medical Entomology, Calcutta School of Tropical Medicine, Calcutta, India, 700073.

Japanese encephalitis (JE) first brokeout in epidemic form in West Bengal in 1973. JE virus was isolated thrice in nature during 1973 to 1982 (1973, 1976 & 1982) from wild caught Culex vishnui. From a longitudinal study, conducted for three years, it was quantified that per man hour density of C.vishnui of above 7.0 in human habitations was able to transmit the disease in man in favourite season. There was an association between the density of the vector, the incidence of infections in the sentinel chicks, exposed at monthly intervals, the prevalence of antibodies in wild birds and the occurrence of clinical infections in the human population. Fluctuations in the level of circulation of the virus in the maintenance cycle and spillover to the human population at times of peak circulation was observed.

R10.30

DISCONTINUOUS AND CONTINUOUS EPITOPIC SITES IN THE DENGUE-2 VIRION PROTEINS. R. Feighny, M. Burrous, M. Gentry, C. Hoke, K. Kopec, and D. Burke. Walter Reed Army Institute of Research, Washington, D.C.

We have purified the envelope glycoprotein of the dengue-2 virus to homogeneity. Monoclonal antibodies have enabled us to identify four antigenic regions on the protein, designated IA, IB, II, and III. Three regions, IA, IB, and III, react with neutralizing monoclonal antibodies. Regions IA and IB are very sensitive to disulfide bond cleavage and denaturation and are therefore presumed to be dependent on the secondary and tertiary conformation of the protein. Region III is relatively stable under these conditions and is probably defined by the primary amino acid sequence. The reactivity of different peptides generated in tryptic digests toward different monoclonal antibodies is further indication that the IA and IB regions are discontinuous epitopic sites, whereas the III region, which reacted only with monoclones in a single group, is a continuous site. Site IB is reactive toward monoclonal antibodies which are involved in immune enhancement of viral replication in vitro. The separation and characterization of neutralizing sites from enhancing sites suggests that sub-unit vaccines may be developed which stimulate antibodies which protect but do not enhance replication. Site III would appear to possess the most desirable characteristics for a vaccine.

R10.31

Complete Nucleotide Sequence of Japanese Encephalitis Virus Genome RNA. Hideo Sumiyoshi, Chisato Mori, Isao Fuke, Kooichi Morita, Jyun Kondou, Yoh Kikuchi, Hiroshi Nagamatu, Tadayoshi Shiba, Yoshiyuki Sakaki and Akira Igarashi
Department of Preventive Medicine and of Virology, Institute of Tropical Medicine, Nagasaki University, Nagasaki 852 Japan.

Complete nucleotide sequence of Japanese encephalitise virus (JEV) genome RNA has been determined by cDNA cloning and DNA sequencing and by direct RNA sequencing. The JEV genome consist of 10,976 nucleotides and contained a single long open reading frame of 10,296 nucleotides. This long polypeptide is supposed to be cleaved into three structural proteins and sevral nonstructural proteins of the virus. The location of the three structural proteins were determined by the analysis of N-terminal amino acid sequences of three structural proteins. A comparison of the deduced amino acid sequence with those of RNA-dependent RNA polymerases of other RNA viruses indicated significant sequence homology was found in the open reading frame of the 3' region JEV RNA. With Flavivirus RNA, two distinct secondary structures could be formed and the functional implications of thire structural features are discussed.

R10.32

MOLECULAR CLONING AND SEQUENCING OF THE COMPLETE GENOME OF DENGUE 2 VIRUS, JAMAICA GENOTYPE. V. Deubel, R. M. Kinney, D. W. Trent. Division of Vector-Borne Viral Diseases, Centers for Disease Control, Ft. Collins, Colorado, USA.

Dengue 2 (DEN-2) virus, one of the four dengue virus serotypes in the flavivirus genus, contains a 11 kb single-stranded, positive sense RNA genome. Five cDNA clones, ranging from 1.5 to 4.2 kb in length, were constructed using specific oligonucleotide primers for first strand cDNA synthesis from genomic RNA template. Preliminary sequencing and hybridization studies indicated that these cDNA clones contain all of the sequence information in the RNA genome. The DEN-2 RNA contains one long open reading frame of 10.2 kb, beginning with the first AUG codon located 96 nucleotides from the 5' end of the genome. The organization of the DEN-2 genome is similar to that of the other flaviviruses. Putative cleavage points of the translated DEN-2 polyprotein were selected by homology with the polyproteins of yellow fever (YF) and West Nile (WN) flaviviruses. The polypeptides of DEN-2 virus showed 50% to 97% homology with cognate protein sequences reported for other DEN viruses and 13% to 65% homology with WN and YF viral polypeptides. Amino acid conservation was greatest among the respective envelope, NS1, NS3, and NS5 proteins of these flaviviruses. Regions of most extensive homology may indicate possible group-reactive flavivirus antigenic determinants or areas critical to biological function.

R10.33

THE ENTRY MODE OF FLAVIVIRUS WEST NILE INTO P388D1 CELLS:THE EFFECT OF pH. T.Kimura & A.Ohyama. Department of Microbiology Kansai Medical University, Moriguchi, Osaka 570, JAPAN.

The entry mode of the flavivirus West Nile(WNV) into cells of the macrophage-like cell line, P388D1, was assayed by transmission(TEM) and scanning(SEM) electron microscopies, by following the association of Pronase-resistant ^{35}S-labelled virus and by using a radiobinding assay with an ^{125}I-labelled F(ab')$_2$ fragment of a monoclonal antibody directed against the major viral envelope surface protein(E). Using ^{35}S-WNV, virus particles were shown to have an increased affinity for the cell structure at pH below 7.0. The TEM-study showed that this was because WNV was fusing onto P388D1 cells in a pH-dependent manner with maximum fusion occuring at pH 6.4 or below. The high resolution SEM clearly supported the above finding in three dimensions. The monoclonal antibody fragment had an increased affinity for cell surface E protein after prebound WNV was warmed at mild acidic pH values. The protease cleavage experiment suggested that a relevant acid-induced conformational change at E molecule results in fusion with a consequent increase in association of virus and an increase in the recognition of cell surface-expressed viral E antigen by labelled antibody. Electron microscopy and radiobinding studies showed a balance exists between endocytosis and fusion of WNV with the P388D1 plasma membrane, with pH of extracellular medium deciding which one predominates.

R10.34

THE IN VITRO SYNTHESIS OF FLAVIVIRUS SPECIFIC RNA.
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Virus-specific RNA-dependent RNA polymerase activity was detected in a cell-free extracts from Vero cells infected with Japanese encephalitis(JE) or dengue virus. Enzyme activity was not detected in uninfected cell extracts. Examination on the distribution of virus-specific RNA polymerase by the differential centrifugation at 1,000g and 10,000g indicated that most of the enzyme activity remained in the pellet fractions. This suggests that flavivirus specific RNA polymerase was attached to membrane fractions. RNA synthesis in the in vitro system required all four ribonucleotide triphosphates and magnesium ions. The reaction at 30℃ proceeded linearly for about 15min. RNA synthesized in vitro had similar electrophoretic mobility to JE genomic RNA. After the treatment with trypsin, RNA polymerase activity in the crude extracts was not detected. NP40 at 1% and actinomycin D at 5ug/ml did not have any effect to the polymerase activity. This in vitro RNA synthesis system seems to be useful to analyze the mechanism of flavivirus RNA replication.

R10.35

COMPARISON OF NEUTRALIZING ANTIBODY TITERS IN SERA FROM PATIENTS WITH JAPANESE ENCEPHALITIS (JE) AND FROM CHILDREN VACCINATED WITH JE VACCINE. Y. Okamoto[1], Y. Okuno[1], A. Yamada[2]. [1]Department of Preventive Medicine, Research Institute for Microbial Diseases, Osaka University, Yamadaoka, Suita, Osaka 565, Japan, [2]Department of Microbiology, Kyoto Prefectural University of Medicine, Kyoto, Japan.

Several serum samples obtained from patients with JE and those from children vaccinated with current inactivated JE vaccine were studied by the neutralization (N) test. Eight JE virus strains which had been isolated in Japan, China and Thailand were used for the N test, and they were propagated in suckling mouse brains (SMB) or mosquito cells (C6/36 cell) as stock viruses.

All the sera showed significantly higher N titers to the C6/36 cell-passaged viruses than to the SMB-passaged viruses. Differences of N titers among JE virus strains were small. However, only to the Nakayama-Yoken strain, sera from vaccinees showed considerably higher N titers to the SMB-passaged virus than to the C6/36 cell-passaged virus. The result seems to reflect the properties of JE vaccine because it is produced from mouse brains infected with the Nakayama-Yoken strain.

R10.36

BLOCKADING OF MACROPHAGES AND ANTIBODY DEPENDENT ENHANCEMENT OF YELLOW FEVER VIRUS INFECTION IN VIVO
by C.A.GIBSON[1], A.Pryde[1], A.R. Madlan[1], M.J. Langley[1], [2]P.D. Minor and A.D.T. Barrett[1]. [1]Dept. of Microbiology, University of Surrey, Guildford, GU2 5XH, UK and [2]Division of Viral Products National Institute of Biological Standards and Control, London, NW3 6RB.

Antibody dependent enhancement (ADE) of viral infectivity is a well established phenomenon in some flavivirus infections, (e.g. Dengue shock Syndrom). However, previously only in-vitro models have existed for studying ADE. We report the development of an adult mouse model system capable of demonstrating ADE of yellow fever virus in vivo.

We have examined the virulence of African and S.American wild-type and vaccine strains. Following intracerebral inoculation, all strains proved lethal to mice, whilst several were also lethal following intranasal inoculation. However, using other inoculation routes, all mice were found to survive infection. Blockading of macrophage function with sodium auro thiomalate (SATM) resulted in enhancement of disease virulence and lethal infections following intraperitoneal inoculation of virus. Similarly use of SATM enhanced lethal infection of mice following intracerebral inoculation of <1 LD50 of virus.

Monoclonal antibodies raised against several flaviviruses were also examined in the model system. ADE was demonstrated following infection of either FMV or 17DD vaccine strains. The role of the macrophage in disease enhancement is discussed.

R10.37

STUDIES TOWARDS ANALYSING ATTENUATION AND VISCEROTROPIC DISEASE OF YELLOW FEVER VIRUS. A.D.T. Barrett[1], T.P. MONATH[2], C.B. Cropp[2], A. Pryde[1], C.A. Gibson[1], B.R. Miller[2], R.M. Kinney[2] and D.W. Trent[2]. [1]Department of Microbiology, University of Surrey, Guildford, Surrey, U.K. and [2]Division of Vector-Borne Viral Diseases, Centers for Disease Control, Fort Collins, Colorado, U.S.A.

Studies in the 1960's showed that passaging of the wild-type Asibi virus strain (wild-type parent to the attenuated 17D vaccine strain) of yellow fever virus in Hela cells resulted in the generation of a virus which failed to kill monkeys or cause viscerotropic (i.e. liver) disease. These experiments have been repeated using a biochemically and biologically characterised plaque purified Asibi virus.

The Asibi virus present after six passages in Hela cells has been biologically and biochemically characterised and compared to (1) Asibi virus with no Hela passages and (2) the attenuated 17D vaccine virus strain. Results show that there are biological differences between the attenuated and virulent viruses and studies with monoclonal antibodies show antigenic differences between attenuated and virulent viruses.

Correlation of biological and biochemical properties with viscerotropic disease and attenuation will be discussed with the aim of determining the molecular basis of attenuation and virulence of yellow fever virus at the nucleotide level.

R10.38

ANALYSIS OF YELLOW FEVER VIRUS ENVELOPE GLYCOPROTEIN EPITOPES WITH MONOCLONAL ANTIBODIES.
by A. PRYDE,[1] A.R. Hedlan,[1] J.J. Schlesinger[2], C.A. Gibson[1], P.D. Minor[3]and A.D.T. Barrett[1]. [1]Department of Microbiology, University of Surrey, Guildford, GU2 5XH, U.K., [2]Infectious Diseases Unit, Department of Medicine, University of Rochester School of Medicine, Rochester, New York, 14021, USA and [3]Division of Viral Products, National Institute of Biological Standards and Control, London, NW3 6RB, UK.

Monoclonal antibodies (MAbs) were prepared against 3 yellow fever 17D vaccines : 17D-204 manufactured in the USA., the 17D-204 World Health Organisation Avian leukosis free seed virus and 17DD manufactured in Brazil. The MAbs were used to compare epitopes present on the envelope glycoprotein of 17D vaccines (both 17D-204 and 17DD substrains) with those present on the French Neurotropic Vaccine and Asibi virus, the wild-type parent to the 17D vaccine. In these experiments antigenic differences between the viruses were identified. In addition, the MAbs have been used to examine epitopes involved protective immunity (ie, passive protection of mice and plaque reduction neutralisation tests). Although the viruses examined shared epitopes the biological properties of these epitopes were found to vary depending on the virus being analysed. The results obtained will be discussed in terms of relating antigenic changes with attenuation and virulence of yellow fever virus.

R10.39

THE NEUTRALIZATION SITE OF VENEZUELAN EQUINE ENCEPHALITIS VIRUS. S. Johnson, J. Brubaker, J. Roehrig, R. Kinney, and D. Trent.
Division of Vector-Borne Viral Diseases, Centers for Disease Control, Ft. Collins, Colorado U.S.A.

The E2 glycoprotein of Venezuelan equine encephalitis (VEE) virus elicits antiviral neutralizing (N) antibody. Monoclonal antibodies (MAb) to epitope $E2^C$ neutralize viral infectivity, block viral hemagglutination, and passively protect mice against virulent VEE virus challenge. Other E2 epitopes which evoke N MAbs are spatially proximal to the $E2^C$ epitope, as demonstrated by competitive binding assay. MAbs to the E2 viral glycoprotein were used to select neutralization escape variants of TC-83 VEE virus. Stable antigenic variants were screened by ELISA for their ability to bind each of 9 MAbs. Variant viruses which escaped neutralization lost the ability to bind their selecting MAb; binding of other MAbs whose eliciting epitopes are spatially close to that of the selecting MAb was lost or depressed. The nucleotide sequence of the genes encoding the E2 and E1 glycoproteins was determined for 3 mutants. A single nucleotide change was identified in the E2 gene of each variant. The mutations affected amino acids E2 182, 183, and 199, producing Ser $\rightarrow$ Arg, Gly $\rightarrow$ Val, and Glu $\rightarrow$ Lys substitutions, respectively. These mutations define a region which will be modeled by synthetic peptides and evaluated as a vaccine.

R10.40

SEROLOGICAL STUDIES ON A JAPANESE CHILD WHO CONTRACTED DENGUE HEMORRHAGIC FEVER (DHF). Y. Okuno[1], T. Harada[2], Y. Okamoto[1]. [1]Department of Preventive Medicine, Research Institute for Microbial Diseases, Osaka University, Suita, Osaka 565, Japan, [2]Department of Pediatrics, Itami City Hospital, Koyaike, Itami, Hyogo, Japan.

In 1986, a Japanese girl (6 year old) contracted febrile illness and developed peripheral cyanosis followed by hemorrhagic manifestations (epistaxis, petechiae, etc.) while she was traveling in Indonesia. Her father who accompanied her also had fever in the same time of her illness. Their bloods were taken and studied serologically.

Her convalescent phase sera showed high hemagglutination-inhibition (HI) titers (x5120) to all the dengue types and Japanese encephalitis (JE) virus. Even by the neutralization (N) test storong cross-reactivity among dengue types was observed although highest titer was shown to dengue type 1. However, her father's serum was clearly demonstrated to have highest titer to dengue type 1. As this was her first trip for abroad and she had been vaccinated with JE vaccine before, her case was primary dengue infection with type 1 which developed secondary antibody response due to previous immunization with JE vaccine.

R10.41

CHANGES IN CEREBRAL LEVELS OF MONOAMINES THROUGH DEVELOPMENT OF FLAVIVIRAL ENCEPHALITIS. Y.Yamashita[1], T.Ito[1], A.Ohyama[1]and K.Kuriyama[2] Dept. Microbiol.[1], Kansai Med. Univ., Moriguchi, Osaka 570, Dept. Pharmacol.[2], Kyoto Pref. Univ. Med., Kamikyo-ku, Kyoto 602, JAPAN.

Alterations in the cerebral levels of dopamine(DA), norepinephrine(NE) and serotonin(5-HT) following viral replications in the brain were studied to elucidate the pathophysiology of viral infection to the central nervous system using experimental models of Japanese encephalitis virus(JEV) and Dengue virus(DV)-induced encephalitis in mice. Monoamine concentrations were measured by high performance liquid chromatography with electrochemical detection and viral replication was detected by immunohistochemistry. 1. In the brain having JEV inoculation, the levels of DA showed a significant decrease, while that of NE exhibited a tendency of increase. Similary, the cerebral content of 5-HT showed a decrease. The viral antigen was detected in the cerebral cortex, hippocampus, mesencephalon and diencephalon in addition to the substantia nigra and striatum. In the latter tissues, the antigen positive cells were detected with the highest density in the brain. 2. In the DV inoculated brain, the level of DA exhibited a significant decrease. The viral antigen was also detected in the cortex, hippocampus, substantia nigra, Purkinje cell layer and striatum. From these results, it is presumed that clinical manifestation of JEV and DV infections may involve the changes in the metabolism of neurotransmitter, especially that of DA in the brain.

R10.42

FAILURE OF DENGUE SUB-UNIT VACCINE TO PROTECT AGAINST LETHAL INFECTION IN MICE. J.G. Aaskov, J.A. Fletcher, S. Wedgwood and R. Hay. Queensland Institute of Medical Research and Queensland Institute of Technology, Brisbane, Australia.

Because of the potential problems associated with the use of live dengue vaccines we evaluated the efficacy of a dengue structural protein in eliciting a protective immune response in mice. We chose the pre-M protein because many dengue patients produce antibody against it; there are common antigenic epitopes in the primary sequences of at least dengue 1,2 and 3; this protein is not known to be involved in antibody mediated enhancement of dengue infection.

Female Balb c mice were primed i.p. with dengue 1 (HAW) pure pre-M protein in Freunds complete adjuvant and challenged 4 and 8 weeks later with pure pre-M in PBS. Immediately following the last challenge the mice were mated.

At birth some mice from each litter were sacrificed to provide serum while remaining newborns were infected i.c. with 100 mouse LD_{50} of dengue 1,2,3 or 4. Mice born to untreated mothers acted as controls.

Serum from immunized mothers and their litters reacted with reduced and non-reduced pre-M and M protein in western blots. They also reacted by immunofluorescence with dengue 1-4 infected C6-36 cells. Despite this, none of the new born mice showed any resistance to the i.c. challenge with live virus.

R10.43

STRUCTURE OF THE DENGUE-1 (HAWAII) GENOME AND EXPRESSION OF E AND NS1 ANTIGENS IN *E.coli* P. W. Mason[1], P. C. McAda[2], M. Zeugel, T. L. Mason and M. J. Fournier. Department of Biochemistry, University of Massachusetts, Amherst, MA 01003 USA, [1]Present address, Yale Arbovirus Research Unit, Yale University School of Medicine, New Haven, CT 06510 USA, [2]Present address, Panlabs Research, 1550 North 115th, Seattle, WA 98133 USA.

A DEN-1 cDNA library was obtained by reverse transcription of the viral RNA and cloning of the resulting ds-cDNA fragments into pBR322. The cDNAs were subcloned into λgt11 and segments encoding NS1 antigen were identified by screening of λgt11 plaques with antisera to DEN-1. Flanking DNA regions were then isolated by cross-hybridization assays and ordered by restriction analysis. The 5' half of the genome is represented by four overlapping clones. DNA sequencing and expression assays of the region encoding C-M-E-NS1-ns2 show the DEN-1 genome to be organized like that of the other sequenced flavivirus genomes. Recombinant E and NS1 proteins have been developed in *E.coli* with a *trpE* expression system. The E fusion protein contains 412 (45.2 kDa) of the 503 amino acid residues of the mature E protein. The NS1 fusion includes 395 residues (44.9 kDa) of the NS1-ns2a region, including 355 C-terminal residues of NS1. The fusion proteins are effective immunogens in mice and provide good binding targets for anti-dengue MAbs. The epitopes for both E and NS1 MAbs are being mapped through deletion analysis and *in vitro* mutagenesis.

R10.44

IMMUNOLOGICAL PROPERTIES OF JAPANESE ENCEPHALITIS VIRUS ANTIGENS EXPRESSED IN *E.COLI*. P. W. Mason, M. J. Fournier, and T. L. Mason. Dept. of Biochemistry, Univ. of Massachusetts, Amherst, MA 01003, USA, Present address, Yale Arbovirus Research Unit, Yale Univ. School of Medicine, New Haven, CT 06510, USA

Several *E.coli* plasmids have been constructed that efficiently express different segments of the JE (Nakayama) polyprotein in the form of fusions with the *E.coli* TRPE protein. The following protein sequences have been expressed: residues 280-414 and 97-500 of E, 58-414 of NS1, 1-100 of ns2a, approximately 190 residues of ns4a, 510 residues spanning ns4a-ns4b and part of NS5, and approximately 380 residues of NS5. All of the purified fusion proteins except the one for ns2a elicited anti-JE antibodies in mice. The specificity of the resulting antisera was examined by western blot analysis with antigen from JE-infected C6/36 mosquito cells. The sera raised against the E and NS5 constructs showed the expected reactivities. Surprisingly, however, the anti-NS1 sera reacted strongly with NS1 from JE-Nakayama but not with the NS1 produced by another JE isolate. Apparently the immune response to the NS1 fusion protein targets a variable antigenic determinant within NS1. Antibodies against the ns4a fusion reacted strongly with a 24 kDa polypeptide (probably NVX) in the infected cell lysates, identifying this polypeptide as a product of the ns4a region of the JE genome.

R10.45

PROTEOLYTIC PROCESSING OF THE NONSTRUCTURAL PROTEINS OF SINDBIS VIRUS AS ELUCIDATED USING MONOSPECIFIC ANTIBODIES. W. Reef Hardy, James H. Strauss. Division of Biology, California Institute of Technology, Pasadena, CA 91125.

The nonstructural proteins of Sindbis virus are translated as a large polyprotein precursor which is subsequently processed to produce four mature proteins. To better understand the kinetics of processing of these proteins and their assembly into functional replicase complexes we have generated monospecific antibodies to each of the proteins to use as a probe. By using the trp E expression system in E. coli and a cDNA clone of Sindbis virus we generated fusion proteins from constructs containing a majority of the sequence of each nonstructural protein gene fused to the N-terminal end two-thirds of the trp E gene of E. coli. Antibodies were produced to these fusion protein antigens, and this antisera was used to immunoprecipitate the nonstructural proteins from cells infected with a heat-resistant small plaque strain as well as several temperature-sensitive conditional lethal mutants. From our data we suggest a model for the processing of the nonstructural proteins of Sindbis.

R10.46

SEQUENCE AND EPITOPE ANALYSIS OF JAPANESE ENCEPHALITIS VIRUS GLYCOPROTEIN E. K. Yasui, J. Kimura-Kuroda, M. Ogimoto, M. Miyamoto. Dep. of Microbiol., Tokyo Metropolitan Inst. for Neurosci. Fuchu, Tokyo, JAPAN.

To make epitope map on the E protein according to amino acid sequence, we cloned molecularly Japanese encephalitis virus(JEV) genome RNA and sequenced. And the E proteins expressed from the cloned cDNAs were analysed the antigenicity by monoclonal antibodies against JEV E protein.

The amino acid sequence of JEV E protein was deduced from the base sequence analysis and compared with those of other flaviviruses. There was a remarkable degree of amino acid sequence homology in the E proteins among serologically related JEV, MVEV, WNV, and SLEV, whereas the serologically more distinct YFV and DENV showed less homology with JEV. Three highly conserved amino acid sequence regions among flaviviruses were observed. Cysteine residues were highly conserved among 6 flaviviruses and might have important role for stabilising the three-dimentional structure of the E protein. Full length and some part of the E protein were expressed or in vitro translated by mRNA transcrived from the clone cDNAs and analysed by using monoclonal antibodies and anti E serum. From the results we will discuss on the epitope arrengement on the E protein.

R10.47

ANALYSIS OF ANTIGENIC VARIATION OF JAPANESE ENCEPHALITIS VIRUS STRAINS USING MONOCLONAL ANTIBODIES. N. Kobayashi, J. Kimura-Kuroda*, Y. Nomura and K. Yasui*. Yokohama City Institute of Health, *Microbiol., Tokyo Metropolitan Institute for Neurosciences, JAPAN.

To analyse antigenic variation among Japanese encephalitis virus (JEV) strains, more than 70 isolated from human, animal and mosquitoes in Japan and ohter Asian countries were investigated their reactivities with monoclonal antibodies (MAbs) against JEV glycoprotein E. Flavivirus cross-reactive, JEV specific and JEV strain-specific MAbs were used in this assay. It was shown by competitive binding assay that the epitopes of strain specific MAbs might be adjacent to the site of JEV specific MAb, which show high neutralising activity and protective activity of JEV infection on mice.

The JEV specific epitope was highly concerved in all JEV strains tested. Seventy two strains tested could be classified into four types from their reactive-patterns with strain specific MAbs, and all four strain types have been still isolated. But in Yokohama area, eastern part of Japan, only one type (Yokohama type) have been isolated since 1960.

R10.48

SITE-DIRECTED MUTAGENESIS OF THE PROPOSED CATALYTIC AMINO ACIDS OF THE SINDBIS VIRUS CAPSID PROTEIN AUTOPROTEASE. Chang S. Hahn, James H. Strauss. Division of Biology, California Institute of Technology, Pasadena, CA 91125.

Current data suggests that the precursor of the structural proteins of the alphaviruses is processed by an autoprotease activity that maps in the C-terminal domain of the capsid protein. We have previously postulated that His-141, Asp-147, and Ser-215 of the Sindbis virus capsid protein form a catalytic triad similar to that found in mammalian serine proteases. To test this hypothesis, each of these amino acids has been replaced with other amino acids using oligonucleotide site-directed mutagenesis: Ser-215 has been changed to Thr, Ile or Cys; His-141 to Pro; and Asp-147 to His or Tyr. Each of these mutagenized plasmids which contain the entire sequence of 26S subgenomic mRNA adjacent to the T7 promoter was used as template for in vitro transcription using T7 polymerase and was followed by in vitro translation using rabbit reticulocyte translation system.

The Ser to Cys mutant retained more than 70% of activity (percentage of the cleaved products) and the Ser to Thr mutant retained up to 10% activity. The Ser to Ile and His to Pro mutants lost the protease activity completely. However, the Asp to His and Asp to Tyr mutants retained 100% activity. Although most of the mutants retained the activity, none of these mutants produce the virus.

R10.49

EPITOPE MAPPING OF TBE VIRUS GLYCOPROTEINS

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The antigenic structure of the E glycoprotein of Tick-borne encephalitis (TBE)virus was studied using a panel of monoclonal antibodies (Mabs). At least 14 epitopes were identified which have different properties with respect to serological reactivity, funcitional activity or topological localisation.

They were found to cluster in three nonoverlapping antigenic domains, A, B, and C.

Most epitopes of domain A are denaturation sensitive. Low pH-induced conformational changes were localized to certain epitopes within domain A. These may play an important role for membrane fusion events in the course of virus infection. The B domain epitopes are also highly conformation-dependent, but their structure is stabilized by disulfide bridges as revealed by reduction and carboxymethylation experiments. These disulfide bridges are probably also responsible for the protease resistant tertiary structure of domain B.

R10.50

SUSCEPTIBILITY AND TRANSMISSIBILITY OF AEDES AEGYPTI TO FOUR STRAINS OF CHIKUNGUNYA VIRUS. D.T. Mourya and K. Banerjee (National Institute of Virology, Pune, India).

The susceptibility of colonized Aedes aegypti mosquitoes to four strains of chikungunya virus was studied by infecting them parenterally. Three virus strains were isolated during epidemics at Madras, Calcutta and Nagpur from India and one was from Africa.

The Calcutta strain apparently was more virulent than the other strains, for, proportionately larger number of mosquitoes transmitted the virus to infant mice. This strain also showed a higher probability of producing illness in infant mice by infective-bite as compared with the other strains.

The susceptibility and transmission potentials of the different strains of virus were different from each other. The virus multiplied slower in the Nagpur strain.

R10.51

OLIGONUCLEOTIDE FINGERPRINTING OF STRAINS OF JAPANESE ENCEPHALITIS VIRUS. S.N. Ranadive and K. Banerjee (National Institute of Virology, Pune, India).

Japanese encephalitis (JE) has emerged as a major public health problem in India. Several strains of the virus have been isolated from different parts of the country in different years.

Oligonucleotide fingerprinting is a powerful tool in the study of different virus strains of the same serotype. RNA from 7 strains of Indian origin, 2 strains of Japan, 1 of Sri Lanka and 1 of China have been fingerprinted. The similarity ratios (SR) were calculated from the different spots. Considering the Nakayama strain as standard (SR = 1.0), the Beijing strain had SR 0.79 and the Tirunelveili (Tamil Nadu, S. India) had SR 0.75 while the West Bengal and the Assam strains had SRs 0.35 and 0.39 respectively. The JaGAR-1 strain closely related to the Bhopal strain (SR 0.98) but far removed from the West Bengal and Assam strains (SR 0.23-0.31). A detailed analysis will be presented.

R10.52

GENETIC DETERMINANTS OF ROSS RIVER VIRUS VIRULENCE

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To examine the antigenic structure of the alphavirus Ross River virus (RRV), RRV T48 antigenic variants were selected with neutralizing mAbs. Each showed a non-conservative amino acid change in glycoprotein E2 at residues 216, 234 or 246-251 defining three epitopes (a, b1 and b2). This region of E2 is not strongly conserved in sequence between alphaviruses but is conserved in its hydropathy profile suggesting a common conformation. In experiments on viral penetration, epitope b2 mutants showed a difference in rate of acquisition of resistance to neutralization by added polyclonal antibody compared to wild type virus, suggesting a role for the neutralization domain in viral entry.

In the course of a single 7 day passage of a mouse-avirulent strain (RRV NB5092) in infant mice, there was a 10^4- fold decrease in LD_{50} for infant mice. To examine the relationship between this increase in virulence and alterations in viral structural proteins, sequencing studies were carried out. There were no changes in the structural protein genes of 2 plaque purified isolates obtained after such passaging. Following 8-10 serial passages, virus with changes in E2 was selected; limited changes in the non-structural protein genes were also observed.

R10.53

IN VIVO PROTECTION STUDIES USING FLAVIVIRUS GROUP SPECIFIC MONO-CLONAL ANTIBODIES. U. Olshevsky, S. Lustig, B.Lachmi, V. Akov and N. Epstein.
Israel Institute for Biological Research, Ness-Ziona, Israel.

The protective ability of West Nile (WN) virus specific, and flavivirus group specific mono-clonal antibodies (Mc Abs) was studied. MC Abs were injected intraperitoneal (ip) or intravenously (iv), to mice, one hour or twenty four hours prior to WN virus challenge. Both types of MC Abs were protective against ip virus challenge, but not against intracerebral (ic) inoculation of the virus.

Studies of the immune response of the protected mice, indicated that while WN specific MC Abs prevented any detectable replication of the infecting virus, some virus replication took place under flavivirus group specific MC Abs protection.

R10.54

CHARACTERIZATION OF FIVE EPITOPES ON THE ENVELOPE-PROTEIN OF WEST NILE VIRUS. USING MONO-CLONAL ANTIBODIES.

B. Lachmi, N. Epstein and U. Olshevsky.
Israel Institute for Biological Research, Ness-Ziona, Israel.

Twelve mouse mono-clonal antibodies (MC Abs) directed against the envelope-protein (E protein) of West Nile (WN) virus, were tested in vitro using the following methods:

1) In vitro nutralization of WN virus.
2) Haemaglutination Inhibition (HI) with WN and 17 D antigens.
3) Enzyme linked immuno-sorbet assay (ELISA) on WN virions.

Five different epitopes were identified on the WN E-protein, based on the reactivity of the MC Abs:

1) WN specific-high NT and high HI activity.
2) WN specific - low NT and high HI activity.
3) Flavivirus group specific - low NT and high HI activity.
4) Flavivirus group specific - no NT but high HI activity.
5) No NT and low HI activity (high activity in ELISA).

R10.55

EXPRESSION OF THE ENVELOPE GLYCOPROTEIN OF YELLOW FEVER VIRUS BY A SV40 RECOMBINANT VIRUS.

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The complete genome of Yellow Fever (YF) virus (17 D 204 Pqsteur strain) has been cloned into pBR327. The nucleotide sequence of the 5' region which codes for structural proteins and the first non structural protein NS1 is the same as that reported by Rice et al.(Science,1985) indicating a remarkable stability within these two vaccine strains. A cDNA fragment coding for the envelope glycoprotein E was inserted into a SV40 late expression vector in place of the normal SV40 VP1 gene. The recombinant virus produced by SV1 cells was tested for expression of the YF glycoprotein in infected CV1 cells by immunofluorescence and immunoprecipitation using specific polyclonal and monoclonal antibodies.

In the first construction containing the gene coding for the E protein, and representing the exact sequence of the mature protein, the envelope protein was efficiently synthetized but revealed an unexpected nuclear fluorescence. In the second construction, the 5' end was modified by addition of a restriction fragment corresponding to the 3' end of the H gene and coding for a hydrophobic signal sequence, which has been proposed to induce the envelope protein translocation. This new chimeric gene, containing the putative signal peptide, is being assayed for expression of the viral glycoprotein and its translocation .

R10.56

Comparison of nucleotide and amino acid sequence between E protein gene of several Japanese Encephalitis Virus strains. Hideo Sumiyoshi, Isao Fuke, Chisato Mori, T. Shiba, Yoshiyuki Sakaki and Akira Igarashi
Department of Preventive Medicine and of Virology, Institute of Tropical Medicine, Nagasaki University, Nagasaki 852 Japan.

We have examined the nucleotide sequence of sevral strains of Japanese encephalitis virus isolated from Thailand, China and Japan from different years. cDNA of E protein gene derived from genomic RNA of these strains were cloned and sequenced by using several primers synthesized according to the sequence of strain S982 which has already been determined the entire sequence. The sequence results were compared with each strains. The present findings should be informative to reveal the evolution and the difference in antigenicity of JEV.

R10.57

REGULATION OF THE ANTIBODY RESPONSE TO FLAVIVIRUSES. M.F. Uren, P.C. Doherty, Jane E. Allan. Dept. Experimental Pathology, John Curtin School of Medical Research, Australian National University, Canberra, Australia.

Infection by a flavivirus results in formation of antibody which is cross-reactive for other members of this family. Since the response can be protective, but may also be immunopathological, it is of interest to understand how it is regulated. The numbers of IgG antibody-secreting cells (ASC) in the spleens of mice primed with the flavivirus Kunjin (KUN), Murray Valley encephalitis (MVE) or West Nile (WN) were assayed by the Elispot technique following restimulation of cells in vitro with homologous or heterologous virus. On heterologous stimulation high numbers of ASC resulted from cells primed with WN and restimulated with KUN and also in the reciprocal situation. High numbers of ASC were obtained from populations primed with MVE and stimulated with WN or KUN although fewer ASC were produced by the converse. This pattern is similar to that of polyclonal and clonal $L3T4^+$ T cells.

IgG production required $L3T4^+$ T cells which could be replaced by most of our cloned lines of flavivirus-reactive $L3T4^+$ T cells. T cell clones from WN primed mice stimulated antibody from $L3T4^-$ cell MVE primed cultures. Therefore production of antibody occurs in the presence of cross-reactive T cells.

R11.1

ROTAVIRUS AS AN AETIOLOGICAL AGENT OF ACUTE CHILDHOOD DIARRHOEA IN ILE-IFE, NIGERIA. O. OLUSANYA
College of Health Sciences, Ogun State University, Nigeria.

192 faeces samples from children aged 0-4 years with acute diarrhoea and 50 samples from non-diarrhoeic children (control) all in-patients of Ife University Teaching Hospital were examined for the incidence of Rotavirus by Elisa method.

35 (18.2%) of the 192 diarrhoeic samples and none of the control had the rotavirus. The prevalence varied amongst the aged groups as follows: Age 0-6 months had 3 (8.57%), 6 months - 12 months 17(48.57%); 12 months - 18 months 5 (14.28%); 18 months - 24 months 9 (25.71%); 30 months - 36 months 1 (2.85%). None in 24 months - 30 months and above 36 months. The males and females accounted for 62.9% and 37.1% of the cases respectively. All the samples were collected and examined during the dry season as microbial infections are higher during this period. 26 (74.3%) of the affected children lived in areas without pipe-borne water especially during the dry season and belong to low socio-economic group. It is evident from this study that Rotavirus is a major enteric pathogen in acute childhood diarrhoea in this environment. This has thus highlighted the importance of examining all stool samples from children with acute diarrhoea - routinely for the organisms in our laboratory.

R11.2

EPIDEMIOLOGY OF ROTAVIRUS DIARRHOEA IN MALAYSIA
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Dept. of Genetics & Cellular Biology, Faculty of Science[1] and Dept. of Medical Microbiology, Faculty of Medicine[3], University of Malaya; Dept. of Medical Microbiology, Faculty of Medicine, National University of Malaysia, Kuala Lumpur, Malaysia.

The first comprehensive study of rotavirus diarrhoea in Malaysia was undertaken among children up to the age of six admitted in 1985 into the paediatric wards of two hospitals in Kuala Lumpur. A monthly incidence of 4-37% and two broad annual peaks were observed when stool samples were analysed by ELISA and PAGE. The majority of the electropherotypes observed were of the long type plus a few short types. The electropherotypes could be classified into 8 long and 4 short patterns. One particular long electrophoretic profile circulated throughout the year together with other profiles. Analysis of an outbreak of acute gastroenteritis in a small coastal village in the State of Perak also revealed a predominance of the same long electropherotype and an unusual short electropherotype. No atypical profiles were observed; all the RNA-positive samples showed the tight migration of bands 7-8 characteristic of group A rotaviruses. Data on a prospective survey of rotavirus electropherotypes circulating among domestic animals in Malaysia will also be presented.

R11.3

ROTAVIRUS INFECTION IN IRAQ. PARTIAL CHARACTERISATION OF THE VIRUS AND EPIDEMIOLOGY. Nazal Al-Yassiri & F. Hatif
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Rotavirus infection was detected by the ELISA technique in 34(23%) of 150 infants and young children under three years of age hospitalized with acute gastroenteritis at two hospitals in Baghdad starting February 1985 through October 1985. Rotavirus was found to be the most important pathogen associated with childhood diarrhoea detected in this study.

Rotavirus antibody prevalence in 150 serum samples of different age groups was determined by CIEP. Antibody prevalence was highest(72%) within 1-3 years of age indicating a high rate of infection whether symptomatic or asymptomatic the paediatric age group.

CIEP and CD were compared with the ELISA for sensitivity of detecting rotavirus antigen and antibody in stool and serum samples. CIEP had a sensitivity of 89% and 63% for antigen and antibody detection respectively, while CD was found to have a sensitivity of 72% and 42% for antigen and antibody detection respectively.

A strain of RVV was found to be moderately stable to various chemical and physical agents as measured by the ELISA technique. It lacked haemagglutinins for five species of RBCs.

R11.4

NUCLEIC ACID SEQUENCE ANALYSIS AND HYBRIDIZATION USING cDNA CLONES OF ANTIGENICALLY DISTINCT ROTAVIRUS. J. Eiden, S. Sato, S. Vonderfecht, R. Yolken. Department of Pediatrics, Johns Hopkins University School of Medicine, Baltimore, MD, USA. K. Theil, OARDC, Ohio State University, Wooster, Ohio, USA.

We have previously reported the isolation in Baltimore of an antigenically distinct rotavirus (ADR-B) which is associated with murine and human gastroenteritis. ADR-B cross-reacts with group B rotaviruses (GBR) in serologic assays and nucleic acid hybridizations. We now report the characterization of the genome of ADR-B by nucleic acid sequence analysis and hybridization of cDNA clones with other rotaviruses.

Viral genomic RNA derived from ADR-B was cloned using standard techniques with the plasmid, pBR322. Dot hybridizations and Northern blot hybridizations with cloned cDNA were used to define the relatedness of specific gene segments of ADR-B and other rotaviruses. We found, for example, that the third gene of ADR-B hybridizes specifically with gene 3 of bovine GBR. Nucleic acid sequence analysis of the cDNA clones also confirmed that ADR-B was genetically distinct from standard, group A rotaviruses.

These studies document the relatedness of ADR-B to group B rotavirus from different geographic locations and animal species, and the results further define the genetic differences between ARD-B and group A rotaviruses.

R11.5

SUBGROUP DISTRIBUTION OF PORCINE ROTAVIRUS IN VENEZUELAN FARMS. F. Liprandi, L. Palencia*, P. Ramos*, I. Rodrigues, C. Aponte, J. Esparza. IVIC, Caracas, IIV*, Maracay, Venezuela.

Variation and evolution of porcine rotavirus populations were studied over a year period in 5 herds located in the central region of Venezuela. Fecal samples from diarrheic piglets (1-10 week old), positive for rotavirus (overall incidence in the farms of 24% in 849 samples), were analised with monoclonal antibodies specific for subgroup (SG) I and II determinants. In two herds a complete predominance over the whole year of either SG I or II viruses was observed whereas in the others occurred a prolonged cocirculation of viruses of both subgroups. In each farm one electropherotype (ET) of each SG predominated, with a transient appearance of additional (up to 4) ETs. Analysis of samples collected in the same farms one year after the end of the survey showed a change in both the SG and ET characteristics of the predominant circulating viruses, although in one farm the predominant ET was identical to the one previously observed. These virus populations are currently studied with monoclonal antibodies against the rotavirus strain OSU, to correlate ET and SG distribution to antigenic markers related to serotype specificity.

Supported by IFS grant N° B/903-1.

R11.6

POLIO OUTBREAK IN ALGERIA : EPIDEMIOLOGICAL, VIROLOGICAL AND VACCINAL ASPECTS.

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Department of Virology of the Pasteur Institute of Algiers Sidi-Frdj, Algeria.
Department of Epidemiology of the Algiers University.

During a poliomyelitis outbreak in El-Oued territory (Algeria) 28 cases were diagnosed. All the patients were under 4 years old. The Ratio of females to males was 0.33. No death occured during this epidemic. 25 out the 28 Polio cases were diagnosed by cell culture, and 81 % of them were Polio type 1. The epidemiological survey established that the epidemic was due to the insufficiency of vacci- 1 nal coverage, since the consumption of the antipolio vaccine in the epidemic area had dropped 25 % from 1982 to 1983. 25 % of the 28 Polio cases were well vaccinated Indeed, 5 out the 8 vaccine samples brought from the epidemic area has an insufficient titration of Polio type 1. In comparison with national data, this epidemic confirmed that the nature of the Polio vaccine killed or live is not as essential for controling poliomyelitis as the sanitary protection for a good vaccinal coverage.

R11.7

TRANSMISSIBILITY AND IMMUNOGENICITY OF RHESUS ROTAVIRUS VACCINE MMU-18006. N.Adachi,S.Chiba,Y.Morita, T.Nakao(Department of Pediatrics,Sapporo Medical College,Sapporo)K.Taniguchi,T.Urasawa,S.Urasawa(Department of Hygien and Epidemiology,Sapporo Med. College,Sapporo)A.Z.Kapikian(Laboratory of Infect. Diseases,NIAID,NIH)

We have conducted a small trial of rhesus rotavirus vaccine to observe its transmissibility and immunogenicity.Infants living at the same room in Hokkaido Central Orphanage were divided into 2 groups. Twelve infants were given one dose of vaccine while 11 infants of the controls received placebo.Fecal specimens were collected daily from infants of both groups for 3 weeks.Nine of 12 vaccinees and none of controls were positive for fecal shedding of the vaccine virus.No evidence was available to indicate the transmissibility of the vaccine among infants. Eight of the 12 vaccinees had a significant seroresponse and acquired neutralizing antibody titer over 1:128 against the vaccine's strain. However, concomitant antibody responses against other serotypes of HRV strains were insufficient. Besides the antibodies were gradually decreased within 6 months after inoculation.Thus the vaccine is promissing for prevention of disease caused by HRV-3,but a booster dose of the vaccine or polyvalent vaccine would be necessary to cover whole serotypes of HRVs.

R11.8

GROUP C ROTAVIRUS FOUND IN SOUTH CHINA.Chen Guangmu,Fan Beilian,Hung Tao. Institute of Virology, Chinese Academy of Preventive Medical Sciences, Beijing,China.

A group C rotavirus was found in a small outbreak of acute non-bacterial diarrhoea,in which over 50 adults and children were involved.Three rotavirus isolates were detected from stools collected from three patients aged 35,4 and 1 respectively.The patients had symptoms of abdominal pain followed by watery diarrhoea with no fever. The virus was morphologically indistinguishable from typical rotaviruses and its genome RNA showed an atypical pattern resembling that of previously reported group C rotaviruses. Serologically,the virus cross reacted with neither typical rotavirus nor Adult Diarrhoea Rotavirus(ADRV-a group B rotavirus found in China)as shown by ELISA. The virus is,however,related to a group C rotavirus isolated in Britain(J.Bridger) as revealed by immune electrophoresis.

The investigation is undertaken,detailed results are reported elsewhere.

R11.10

CHARACTERISTICS OF ROTAVIRUS STRAINS ISOLATED IN CUBA. María Teresa Frías, Rosa E. Simeón and J. Cohen. Department of Virology-Immunology, National Centre of Animal and Plant Health (CENSA), Post Box 10, San José de las Lajas, La Habana, Cuba.

Thirty-two rotavirus strains isolated from 0-10-day-old calves and piglets, and 45-day-old chickens were studied. For virus identification negative stain electronic microscopy was performed. Bovine strains were purified in caesium chloride isopycnic gradient for the study of their polypeptidic classes. Viral RNA and proteins were characterized by means of polyacrylamide gel electrophoresis.

RNA revealed 11 segments in all strains regardless of their origen; only motility differences of certain segments between animal species were seen. Bovine strains showed 6 polypeptidic classes with molecular weight of 31 000 - 124 000 dalton.

The obtained rabbit antiserum against bovine rotavirus disclosed a positive reaction when challenged with swine and chicken rotavirus, the existence of an antigenic community between strains of different origen being corroborated.

R11.11

INCURVED DISC FORM PARTICLES ASSOCIATED WITH A BOVINE CORONAVIRUS GROWING IN VERO AND CALF TESTICLE CELL CULTURES. A. Mousse

Ministère de l'Agriculture, Direction de la Qualité, Laboratoire National de Pathologie bovine, Lyon - France

A bovine coronavirus adapted to grow in Vero and calf testicle cell cultures induced a syncytial cytopathic effect in Vero cells where no cytopathic effect was visible in calf testicle.

In thin sections of both infected cell types normal characteristic morphogenesis for coronavirus was observed as well as the formation of reticular membrane bounded tubular inclusions. The tubes were 35 nm in diameter and of variable lengths. Some of these tubes had a lucent central area and electron dense outer contours. Virus synthesis was observed within these inclusions and the particles were either spherical about 80 - 120 nm in diameter or in the form of incurved discs of about 100 nm in diameter. According the plane of the sections the particles appeared either spherical with hollow central area or in the form of rods. Some rods showed the hollow central area. These particles accumulate within reticular distended membranes before being liberated to the outside of the cell. Negative staining of fecal samples and the culture supernatants revealed also particles in the form of incurved discs and curved rods.

R11.12

Some Features of Diarrhoeal Diseases in Heilongjiang Province

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We have investigated the morbidity and mortality in children below 5 years with diarrhoeal diseases using the "30 Cluster Sampling Technique" recommended by WHO in May 1985 -1986.The morbidity was 1.01-1.84 episodes per child per year in Harbin and 1.63 episodes in Qiqihar.The mortality was 1.57-1.75% in Harbin and 2.4% in Qiqihar.All the deaths among the investigated children were not due to diarrhoeal diseases.In 1979-1982 we collected 271 fecal samples from hospitalised patients with diarrhoea diseases in Harbin Childish Hospital.Among viral pathogens,rotavirus was found in 60.1%,adenovirus in 2.95% and small round virus in 1.47%.In spring 1982,epidemic adult diarrhoea first occured in our province.Through bacteriological and virological studies we had not found ordinary bacterical and viral pathogens.At last we found a kind of virus-like particle,the diameter of which was abound 50nm and the kind of particle was found related with convalescent serum of the patients by IEM.Then in 1984 this new rotavirus including epodemic adult diarrhoea was identified in Peking,Harbin, Lanzhou,CHINA.The 50nm is a denatured form of this new rotavirus in the supernatant fluid of the fecal samples.So that these particles is an important marker in EM and IEM for rapid diagnosis of epidemic adult diarrhoea.

R11.13

Studying on Characterization of HAV Strains Isolated in2BS Cells Chen Mei Zheng,Meng Qing Hai,Wang Yuong,YongYuongLi Wu Yu Hua. Heilongjiang Provincal Health and Anti-Epidemic Station, Xiang Fang District, Harbin, China.

In 2BS cells of human fibrolast diploid cell line we directly isolated 4 HAV strains from stool specimens of 4 children patients with HA in 3 epidemic areas. We have constantly propagated into the 9th passge.We identified by IF, IEM, ELISAand so on. The strains were investigated and result showed as following: (1). The incubation proid of the 1th passgeof HAV was 21-33 days and the 5th passge was reduced to 7-10 days, and there was no CPE.(2).We have observated that there were specific flouressecence particles in cytoplasm by IF. (3). Viral particles which diameter is in 27-29nm were congealed and convalescent serum by IEM.(4). The buoyant desity of the strains 1.32-1.41g/cm^3 ($CsCl$). (5). P/N value of ELISA ranged 3.6-20.6 by ABBOTT HAVAB-E kit.(6). Viral Neutralization assary showed: It can be neutralized by specific anti-HAV serum and can not be neutralised by control serum.(7). There were resistant to acid and ether.(8). The infectious titration of these strains were 10^{-5}-10^{-6} $TCID_{50}$/0.1ml. These strains were named HAV/Long-1,HAV/Long-6, HAV/Long-7, HAV/Long-25.

These characterizations accord with HAV. These strains may be candidate strains for HAV vaccine and Diagnosis reagent.

R11.14

Isolation and Preliminary Identification of a Small Round Virus Relevanted with Adult Diarrhoea

LiZhongYuan, WangShuXun, MengQingHai, LoShiRong, KuoDongLing. Heilongjiang Provincal Health and Anti-Epidemic Station, Xiang Fang District, Harbin, China.

In autum of 1984 there was a epidemic adult diarrhoea within a unit in Harbin. There were 174 pateints among 340 staff of the unit. The accidence rate was 51.18%. We collected fecal specimens from typical cases and separated by cell culture. We found a small round virus which diameter was in 27 nm by EM. We have successfully cultivated in HA-104 cells and have had CPE. The virus belonged to RNA type virus. There was a resistance to PH 3.0 and ether. It was not neutralised by anti-poliovirus. Neutralization was taken by acute and convalescent serum of pateints with adult diarrhoea. The result showed: The neutralised antibody titrations of acute phase serum from these pateints with adult diarrhoea were below 1:40, and that of convalescent phase serum of pateints with adult diarrhoea were 1:320. There was more than 8 times antibody increasing.

Our preliminary identification consider that may be a new virus strain relevanted with adult diarrhoea.

R11.15

EPIDEMIOLOGICAL STUDIES OF ROTAVIRUS AND ADENOVIRUS GASTROENTERITIS IN JAPAN

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Department of Pediatrics, Tokyo Kosei Nenkin[1] Hospital and Teikyo University School of Medicine[2], Tokyo; National Children Hospital[3], Tokyo; National Osaka Hospital[4], Osaka

We have studied epidemiology of rotavirus (RV) and adenovirus (AD) gastroenteritis in Japan, especially in Tokyo area for several years.

Electron microscopy, latex agglutination tests, polyacrylamide gel electrophoresis, enzyme immunoassay, neutralization tests and others were used for detection and differentiation. More than three thousand diarrheal samples in children including immunodeficiency were collected from Tokyo, Osaka, Maizuru, Sapporo, Hiroshima, Kurume and Okinawa.

Major electropherotypes and serotypes in both viruses were found in each area. Some changes of them were noted in years. Pararotavirus was not found so far. Clinical manifestations except gastroenteritis were also discussed.

We thank Drs. Y.Agazuma, T.Nishimura, M.Takagi, T. Motohiro, Y. Nagamine, Y.Fujita, T.Togo, Y.Kondo Y.Mizuta, Y.Matsunaga, S.Matsuno and A.Mukoyama

R11.16

IDENTIFICATION OF A HUMAN GROUP A ROTAVIRUS ANTIGENICALLY DISTINCT FROM SUBGROUP I AND II VIRUSES.

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Among 231 stool specimens collected from 3-24 months old children suffering from acute gastroenteritis in Dhaka, Bangladesh, 126 were found to contain rotavirus by EM and different ELISAs. Subgrouping by monoclonal antibodies revealed that 32 rotavirus strains belonged to subgroup I and 75 to subgroup II. Four specimens were found to be a mixture of subgroup I and II, and 8 specimens were not typable due to insufficient antigen content. The subgroup specificity of the remaining 7 rotavirus strains could not be defined. These strains showed a distinct reaction with a monoclonal antibody recognizing a common region on Vp6, but they did not react with Vp6 subgroup I and II specific monoclonal antibodies although they contained as much viral antigen as the subgrouped strains. The examined strains were considered to represent a potentially new subgroup of human group A rotavirus.

R11.17

CHARACTERISTICS OF HUMAN ENTERIC CORONAVIRUS-LIKE PARTICLES.

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Human enteric coronavirus-like particles (CVLPs) from Australian Aborigines were shown not to be coronaviruses from structural polypeptide profile and antigenic comparisons. Structural polypeptide and direct electron microscopic comparisons also showed them to be unlike purified duodenal brush border vesicles.

Three highly purified, antigenically identical CVLP preparations obtained from different Central Australian Aborigines in 1982, 1983 and 1984, and an antigenically different fourth preparation from a Vietnamese immigrant, all gave different polypeptide profiles. The number of polypeptide bands ranged from 38 to 48.

Central Australian CVLPs differed antigenically from CVLPs from India, Kiribati, South Africa and Vietnam. In some cases they also differed antigenically from CVLPs from Aborigines from other areas of Australia.

Evidance of circulating antibody to CVLPs had previously been detected in Australian Aborigines and has now also been detected in sera from other countries.

R11.18

MONOCLONAL ANTIBODIES AND DNA RESTRICTION ENZYME ANALYSIS IN DIAGNOSTICS AND EPIDEMIOLOGY OF ADENOVIRUSES 40 AND 41.

J.C. de Jong, A.C. Wermenbol, K. Bijlsma, G. Veerbeek, T.P.L. Zomerdijk, H.G.A.M. van der Avoort, A.D.M.E. Osterhaus.
Laboratory of Virology, Rijksinstituut voor Volksgezondheid en Milieuhygiëne, Antonie van Leeuwenhoeklaan 9, Bilthoven, The Netherlands.

In neutralization tests, the fastidious adenoviruses type 40 and 41 (Ad40 and Ad41) are closely related. Still, on the grounds of the large differences between their DNA restriction profiles, they are called different serotypes (J.C. de Jong et al, J.Med.Virol.11(1983)215-231). To facilitate typing we developed neutralizing monoclonal antibodies to each of these types.

The DNA of fastidious adenoviruses is variable. By DNA restriction enzyme analysis 11 DNA variants were detected among strains of Ad40, and 24 among strains of Ad41. The DNA patterns can be helpful in microepidemiological studies.

For routine typing by neutralization, two monoclonals were selected which reacted with all the DNA variants of the homologous type, and with none of the DNA variants of the heterologous type. The same monoclonals have been used for construction of an ELISA for detection and typing of fastidious adenoviruses directly in stool specimens. This work takes place under W.H.O. grant no C4/181/198.

R11.19

A MONOCLONAL ANTIBODY TO THE GOTTFRIED STRAIN OF PORCINE ROTAVIRUS WHICH NEUTRALIZES ROTAVIRUS SEROTYPES 3, 4, AND 6.

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The purpose of the present study was to develop neutralizing monoclonal antibodies (Mabs) to the OSU (serotype 5) and Gottfried (serotype 4) strains of porcine rotavirus (RV). These neutralizing Mabs would have potential as reagents for serotyping of porcine RV by enzyme immunoassay. Fluorescent focus neutralization assays identified two hybridomas designated 57-3 and 57-8 which neutralized the homologous Gottfried RV and the heterologous simian (serotype 3) and bovine (serotype 6) strains of RV. These Mab did not neutralize other serotypes of RV including the OSU strain of porcine RV. Ascites fluid prepared with both clones were reactive with the VP7 and not the VP3 glycoprotein of the Gottfried strain of RV as determined by reaction with an OSU x Gottfried reassortant RV. Use of other reassortant RV revealed that the Mabs reacted with only the VP7 of serotype 3 and 6. The results are consistent with the hypothesis that rotaviruses of different serotypes share a common neutralization epitope on the VP7, which is the major neutralization antigen on the surface of rotaviruses.

R11.20

ISOLATION OF ENTERIC ADENOVIRUSES BY CELL CULTURE.

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Department of Pediatrics, Faculty of Medicine, Teikyo University, Infectious Diseases Division, Department of Pediatrics, National Children's Hospital, Tokyo, Japan.

Thirty-four stool specimens were collected from patients with acute gastroenteritis and shown to contain adenovirus by electron microscopy. Fifteen stool specimens were inoculated onto 293, HEp-2, and human embryonic kidney cell cultures. Nine specimens induced cytopathic effect on primary inoculation in 293 cells. By use of DNA restriction enzyme analysis, and neutralization test, eight isolates were identified as enteric adenoviruses 40 and 41.

In an effort to increase the efficacy of primary isolation of fastidious adenoviruses, we attempted 293 cells in suspension for the virus isolation from stool supernatant. By this procedure, twenty-one of 29 isolates were determined as enteric adenoviruses. This assay, followed by DNA restriction analysis for typing proved to be a more sensitive procedure than conventional isolation method using serial subcultures.

R11.21

INCIDENCE OF ROTAVIRUS GASTROENTERITIS AMONG IRAQI CHILDREN

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Central Public Health Laboratory, Baghdad, Iraq.

A total of 900 stool samples (600 samples from patients with acute gastroentritis and 300 samples from control), were collected and examined for the presence of human rota virus (HRV) antigens using ELISA test.

HRV antigens were detected significantly higher among children with acute gastroenteritis (40%) than among controls (4.3%), indicating the high pathogenic potential of HRV.

HRV gastroenteritis was significantly higher among patients under 2 years of age than among patients above 2 years of age.

Males were more commonly affected than females in the ratio 1.9:1; there was no significant difference in incidence of HRV gastroenteritis among patients from different socio-economic classes.

Breast feeding seems to provide no protection against HRV gastroenteritis.

R11.22

IMMUNOMODULATING EFFECT OF COXSACKIEVIRUSES

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The variable degree of immunologic hyporeactivity is related with many viruses. Data about influence of Coxsackieviruses on the host's immune system are insufficient and controversy.

In our experiments BALB/c and random bred mice aged 2-2,5 months were inoculated with Coxsackieviruses A13 and B1. On days 7,21,90 and 180 p.i. spleens of infected and control animals were examined for rosette-forming cells (EAC-RFC)(Zaalberg,1964) and antibody-forming cells (Cunningham, 1965). Viruses caused no manifest clinical signs in the adult mice but changed their immune respose to sheep RBC. These changes depended on the time p.i., strain and sex of mice and persistence of virus. Involvement of cell-mediated immunity was discovered in reaction of macrophage's disappearance from peritoneal exudate. Considerable fluctuation in number of leucocytes and lymphocytes of peripheral blood was observed till day 180 (end of observation period). Modulation of host's immunity is important factor of pathogenecity of Coxsackieviruses.

R11.23

CULTIVATION OF TURKEY CORONAVIRUS ISOLATED FROM DIARRHEIC POULTS. S. Dea, S. Garzon, P. Tijssen. Centre de recherche en médecine comparée. Institut Armand-Frappier, Université du Québec, Laval-des-Rapides, Québec, Canada, H7N 4Z3.

Turkey enteric coronavirus (TEC) isolates were obtained from intestinal contents of diarrheic poults and serially propagated in HRT-18 cells, an established cell line derived from human rectum adeno-carcinoma. All isolates were shown to induce syncytia as early as in the second or third passage provided trypsin was incorporated in the culture medium and incubation was done at 41°C. Viral yields increased to $10^{7.0}$ to $10^{8.2}$ TCID 50 per mL during 5 passages, after which the titre remained stable. Electron microscopic examination of infected cell monolayers using protein-A immunogold conjugates and rabbit primary antibodies to TEC, revealed budding of typical coronavirus particles through intracytoplasmic membranes and accumulation of complete virus in the vesicles. The cell-free virus had the characteristics of TEC: shape, surface projections, buoyant density and hemagglutination of rat erythrocytes. One-day old turkey poults inoculated orally with cell culture-adapted TEC isolates developped mild to severe diarrhea.

R11.24

SERIAL PROPAGATION OF PORCINE GROUP C ROTAVIRUS IN AN ESTABLISHED CELL LINE. L. Saif, L. Terrett, K. Miller. Food Animal Health Res. Prog., Ohio Ag. Res. and Dev. Ctr./OSU, Wooster, Ohio USA.

The Cowden strain of porcine group C rotavirus (PaRV) was adapted to serial passage in an established monkey kidney cell line (MA 104). Key factors in its successful adaptation included: use of PaRV passaged in primary porcine kidney cells as inoculum, use of roller tubes, and addition of pancreatin to the maintenance medium. A cell culture immunofluorescent (CCIF) test was used to quantitate virus at each passage level since possible CPE was obscured by use of pancreatin. The PaRV titers dropped after initial passage into MA 104 cells, but increased thereafter with peak titers evident after 10 passages ($2x10^6$ IF units/ml). Immune electron microscopy (IEM) and genome electropherotyping were used to identify PaRV particles and confirm PaRV ds RNA gel migration patterns, respectively from infected cell culture supernatants. No group A rotavirus was detected in the cultures using the 2 former techniques or a CCIF test. Attempts are underway to adapt the PaRV to stationary cultures and to plaque in MA 104 cells. To our knowledge, this is the first verified report of the serial propagation of an atypical rotavirus in an established cell line.

R11.25

GENOMIC HETEROGENEITY OF HUMAN ROTAVIRUSES FROM INFANTILE DIARRHEA IN ITALY. F.M. Ruggeri, M.L. Marziano, E. Salvatori, A. Tinari, G. Donelli. Laboratorio di Ultrastrutture, Istituto Superiore di Sanità, Rome, Italy.

About one thousand children with acute diarrhea from different cities of Italy were included into the study. Rotavirus infection was diagnosed in as high as 30 % of patients by either ELISA or electron microscopy examination of stools. A significant proportion of viral strains were characterized by polyacrylamide gel electrophoretic analysis of genomic RNA. A high degree of heterogeneity was demonstrated among strains circulating in either different cities or epidemic seasons.

A more detailed picture of the molecular epidemiology of rotavirus infection was defined for the area of Rome where sampling started since winter 1982. A single largely predominant electropherotype of rotavirus was revealed in each epidemic season in that city. The succession of the predominant viral strains appeared to occur abruptly with a periodicity of not less than two years. Even though a different electrophoretic behaviour of genomic segments is not proof of antigenic diversity of viruses, our findings suggest that an antibody-mediated selection of strains may occur in the population. Our results also indicate that short-electropherotype subgroup 1 rotaviruses have a minor, if any, role in the etiology of acute diarrhea in our country.

R11.26

PARTIAL CHARACTERIZATION OF A BOVINE GROUP A ROTAVIRUS WITH A SHORT GENOME ELECTROPHEROTYPE. K. W. Theil. Food Animal Health Research Program, OARDC, The Ohio State University, Wooster, Ohio, 44691 USA.

Genome electropherotypes (GEs) of 39 bovine group A rotaviruses from diarrheic beef and dairy calves were determined in polyacrylamide gels. Thirty-eight had long GEs, but one isolate (ID), from a beef calf, had a short GE. The smallest (11th) segment of the short GE migrated only as far as the 10th segment did in the long GEs. The ID isolate induced diarrhea in 4 gnotobiotic calves experimentally inoculated with it between 5 and 21 days of age. The ID isolate GE remained short after 3 sequential passages in gnotobiotic calves or 19 sequential passages in MA104 cell cultures. Therefore the short GE was a stable characteristic of the ID isolate.

Hyperimmune gnotobiotic calf antiserum to the ID isolate had a homologous neutralization titer of 102,400, but had a titer of <25 when tested against human group A rotavirus S2 isolate (short GE) and turkey group A rotavirus 174 isolate (semi-short GE). Hyperimmune gnotobiotic pig antisera to the S2 and 174 isolates had homologous neutralization titers of ≥12,800 and 6,400 respectively, but had titers of <25 when tested against the ID isolate. Subgroup analysis by ELISA tests using monoclonal antibodies to subgroup I antigen (Mab 255/60) and subgroup II antigen (Mab 631/9) indicated the ID isolate belonged to subgroup I.

R11.27

ENTERIC ADENOVIRUS INFECTIONS IN A RURAL AFRICAN SETTING. C.T.Tiemessen, A.H.Kidd, F. Wegerhoff, F.E. Berkowitz, M.J.Erasmus.
Institute for Virology, Johannesburg, South Africa.

Adenovirus types 40 and 41 have been associated with diarrhoea in infants. Our previous studies indicated that genomic variants of each type exist. Little is known of the natural history of infection in a rural African setting. Stools from 312 infants with diarrhoea and 202 control subjects in the Eastern Transvaal were tested by EM, ELISA and dot-blot hybridisation for the presence of Ad40 or Ad41. 13.8% of patients and 1.5% of controls were found to be shedding one or other type, almost exclusively in the under 12-month age group. Shedding of Ad40 was three times as common as Ad41 shedding. In 39.5% of patients shedding one or other type, diarrhoea lasted 4 to 9 days. Details of our attempts to detect Ad40 and Ad41 in nasopharyngeal aspirates from urban children with diarrhoea, and to study the emergence of new virus strains will also be presented.

R11.28

Restricted transmission of rotaviruses between Guangzhou and Hong Kong. J.S. Tam[1], Zheng Hojian[3], Yan Yongkai[3], S.K. Lo[2], M.H. Ng[1]. Dept. of Microbiology[1], The Chinese University of Hong Kong, Hong Kong. Dept. of Microbiology[2], University of Hong Kong, Hong Kong. Dept. of Microbiology and Immunology[3], Sun Yet San University of Medical Science, Guangzhou, People's Republic of China.

Molecular epidemiological surveillance of human rotavirus (HRV) infection in Hong Kong and Guangzhou was carried out to study the transmission of HRV between the areas. The two cities are situated 200 km apart in the southern coastal area of China with identical ethnic and cultural background. Travel between the cities has been heavy and mainly consist of adult travellers from Hong Kong. Study was done by comparing the RNA electropherotypes of HRV isolated in the two cities between 1983 and 1985. A total of 43 electropherotypes were identified from 418 hospitalized and 66 ambulatory cases of HRV infection in Hong Kong, and 19 were identified from 370 hospitalized cases in Guangzhou. Results showed that HRV in the two cities are genetically diverse and constantly changing yet the respective virus populations of the two cities had evolved separately. Only 6 electropherotypes were common to both cities. Three of these were predominant types found in Guangzhou and were transmitted to Hong Kong when they were most prevalent. One electropherotype in this group caused an outbreak in Hong Kong after transmission. Another type was found to be transmitted from Hong Kong to Guangzhou while two appeared in both cities simultaneously.

R11.29

PREVALENCE OF A NEW SEROTYPE OF GROUP A HUMAN ROTAVIRUSES IN JAPAN. N. Ikegami, K. Akatani, T. Hosaka, H. Ushijima. Clinical Research Institute, Osaka National Hospital, Hosaka Pediatric Clinic, Osaka, Department of Pediatrics, Tokyo Kosei Nenkin Hospital, Tokyo, Japan.

We established a rapid assay method for detection and simultaneous antigenic analysis for serotypes and subgroups of group A human rotaviruses from fecal extracts of diarrheal patients. The method is a biotin-avidin enzyme-linked immunosorbept assay (ELISA), using monoclonal antibodies specific to the individual serotypes (1,2,3 and 4) and the subgroups (I and II).

By use of this method, we carried out the epidemiological study on antigenic variation of group A human rotaviruses in Japan in the past few years, and we detected considerable numbers of subgroup II rotaviruses that were not classified into the four known serotypes. Many of these viruses had similar RNA-PAGE patterns to the F45 isolate and shared a specific neutralizing antigenic site with the F45, that was one of the isolates obtained in Osaka in 1985, and was characterized as a new serotype of the subgroup II rotavirus. The study demonstrated that the serotypes 1 and 4 rotaviruses,in addition to this new serotype, prevailed nation-wide in Japan.

We thank the doctors who kindly provided us with fecal samples.

R11.30

THREE DIMENSIONAL STRUCTURE OF ROTAVIRUS. B. V. V. Prasad, G. J. Wang, J. P. M. Clerx and W. Chiu. Department of Biochemistry, University of Arizona, Tucson, AZ 85721, USA.

Three dimensional structures of intact, double shelled and single shelled rotavirus have been determined to 40A resolution using cryo-electron microscopy and image processing techniques. Cryo-electron microscopy of virus in vitrified solution allows one to avoid the artifacts frequently encountered during conventional negative staining and freeze-drying techniques and hence to obtain reliable structural information. Our three-dimensional image reconstructions clearly demonstrate that the structure of both double and single shelled rotavirus is based on T=13 icosahedral lattice. Careful examination of the platinum shadowed single shelled virions revealed that the lattice is left handed (T=13L). Double shelled Rotavirus has an extremely smooth outer surface in contrast to the bristly surface of single shelled virions. A fascinating feature of the structure at both levels is the presence of 132 large aqueous channels at all six and five coordinated centers. These channels are about 60A in width and about 140A in depth. The width of the channel increases at the surface of inner shell and narrows again. It is speculated that these channels would be actively involved in importing metabolites required for the RNA transcription and exporting the mRNA outside the virus. The proteins on both the shells lie on the local and strict 3-fold axes and extend towards the local and strict 2-fold axes. This research is supported by NIH grant No. RR02252.

R11.31

DETECTION AND CHARACTERIZATION OF GROUP A SWINE ROTAVIRUSES WITH REARRANGED RNA GENOMIC SEGMENTS IN ARGENTINA. R.C. Bellinzoni, N.H. Mattion, O. Burrone, A. Gonzalez, J.L. LaTorre, and E.A. Scodeller. Centro de Virologia Animal (CEVAN) Serrano 661 - 1414 Capital Federal, Argentina.

Swine rotaviruses displaying distinctive electropherotypes were isolated from the feces of diarrhoeic piglets in two swine herds in the province of Buenos Aires, Argentina. In one herd, all samples (40) isolated during one year (Sept. 1985-Sept. 1986) showed abnormal electropherotypes. These samples were classified as group A reactive when assayed by an enzyme-linked immunoassay test (ELISA). In other herds, samples displaying both classical and abnormal electropherotypes were found. These viruses were also Group A ELISA positive. Samples with conventional group A RNA patterns and with atypical patterns were adapted to MA104 cells, and these viruses also retained their genomic RNA pattern and the group A antigen reactivity after cloning by limiting dilution. These atypical viruses were tested in northern blot hybridization assay with cDNA probes obtained from RNA segments 10 and 11 of one bovine rotavirus; all samples showed that segment 11 migrated between segment 6 and 7 in the first herd and between segment 9 and 10 in the second herd. Segment 10 was not rearranged. Analysis of ^{35}S-methionine-labeled polypeptides in 10% PAGE demonstrated a similar protein pattern to those of O.S.U. strain of swine rotavirus.

R11.32

MOLECULAR CHARACTERIZATION OF RABBIT ROTAVIRUSES. T.N. Tanaka, M.E. Conner, D.Y. Graham, M.K. Estes. Departments of Virology and Epidemiology and of Medicine, Baylor College of Medicine, Houston, Texas 77030.

Rabbits have proven to be an ideal model to study rotavirus pathogenesis and immunity. Rabbit rotavirus strains were also the first animal rotaviruses characterized with short RNA electropherotype patterns. We have characterized the double-stranded RNA genome segments and proteins of three rabbit rotaviruses, Ala, C11 and R2. The RNA electropherotypes were a standard short pattern for R2, an atypical short pattern for Ala, and an atypical long pattern for C11. The polypeptide patterns of these viruses were similar to those of the simian rotavirus SA11. We used individual cDNA probes of SA11 genes hybridized to genomic RNA segments, separated by polyacrylamide gel electrophoresis and transferred to filters (Northern blots), to identify cognate genes and determine gene-coding assignments for the rabbit viruses. The gene order of segments 7-11 for each of the rabbit viruses was different from one another and from that of SA11 genes. These differences were possibly due to rearrangements of the RNA sequences within the individual genome segments. Sequence analysis of the individual RNA segments will confirm whether genome rearrangements are the molecular basis for these novel migration patterns.

R11.33

DETECTION AND CHARACTERIZATION OF GROUP B ROTAVIRUSES. M.K. Estes, S. Nakata, J.W. Burns, B.L. Petrie, T.N. Tanaka and D.Y. Graham. Department of Virology and Epidemiology, Baylor College of Medicine, Houston, Texas USA.

Recent epidemics of severe diarrhea affecting both adults and children in China have been caused by human adult diarrhea rotaviruses (ADRV) which are group B rotaviruses. We used immune electron microscopy, ELISA and nucleic acid hybridization assays to compare the antigenic, biochemical and genetic properties of the ADRV with rotaviruses in other groups. We found that technical parameters (type, and pH of stain) of the procedures used to visualize these viruses influenced particle stability and virus detection by electron microscopy. An ELISA developed to detect antibody responses to ADRV was used to test human sera and immunoglobulin pools collected from eight countries to begin to assess the prevalence of such infections. Overall, a low proportion of serum samples had antibody to group B rotavirus, although the same samples contained antibody to group A rotaviruses. Seroconversions were detected in paired serum samples from Chinese patients with group B gastroenteritis. These results suggest that human infection with group B rotaviruses has not been widespread in areas of the world outside China. Monoclonal antibodies developed to the ADRV are now being evaluated for detection and to compare the antigenic relatedness of different strains of ADRV.

R11.34

ANALYSES OF THE SIMIAN ROTAVIRUS SA11 HEMAGGLUTININ. J.W. Burns and M.K. Estes. Department of Virology and Epidemiology, Baylor College of Medicine, Houston, Texas USA.

The outer capsid of simian rotavirus SA11 contains two proteins, VP3 and VP7, which are responsible for inducing antibodies that neutralize virus infectivity in vitro. VP3 is also the hemagglutinin and it plays a role in virus virulence and pathogenesis. Cleavage of VP3 by trypsin has been associated with enhancement of infectivity and virus cultivation in tissue culture. We are analyzing the structural and functional properties of the hemagglutinin. A battery of monoclonal antibodies (MAbs) to SA11 were generated and characterized using plaque-reduction neutralization assays, ELISAs with homologous and heterologous virus strains, western blotting and immune precipitation. Thirteen MAbs to VP3 were purified using HPLC and used for epitope mapping in a competition ELISA. At least three neutralization epitopes and three non-neutralization epitopes were found. Work using these MAbs and a variant of SA11 (that plaques in the absence of protease facilitators and has an altered VP3) is focused on defining the molecular basis of protease-enhanced infectivity.

R11.35

THE EXPRESSION AND CHARACTERIZATION OF ROTAVIRUS PROTEINS USING THE BACULOVIRUS EXPRESSION SYSTEM. S.E. Crawford, B.L. Petrie, W.K. Chan and M.K. Estes. Department of Virology and Epidemiology, Baylor College of Medicine, Houston, Texas USA.

The major capsid (VP6 and VP7) proteins and a nonstructural (NS28) glycoprotein encoded by the simian rotavirus SA11 have been produced using the baculovirus expression system. Antigenic, biochemical, structural and functional properties of these proteins expressed alone or in combination in insect (Spodoptera frugiperda) cells have been compared to those of the authentic proteins from mammalian (MA104) cells infected with SA11. The major inner capsid protein VP6 possessed a native oligomeric structure and was able to spontaneously assemble into inner inner capsid morphologic subunits and virus-like particles. Both glycoproteins were detected associated with microsomal membranes and their patterns of synthesis and glycosylation were similar to those seen in MA104-infected cells. The role and interactions of these proteins in the process of virus capsid assembly is being investigated.

R11.36

SELECTION OF REASSORTANTS FOLLOWING COINFECTION OF MA104 CELLS WITH HUMAN ROTAVIRUSES. R.L. Ward, D.R. Knowlton. J.N. Gamble Institute of Med. Res., Cincinnati, Ohio, USA.

The genome of rotaviruses consists of 11 double-stranded RNA segments. Coinfection of cells with two rotaviruses results in progeny with segments from both parents (reassortants). Reassortant formation may be the primary cause of new rotavirus strains. The purpose of this study was to analyze the selection processes in reassortant formation between human rotaviruses in cell culture and the mechanisms that regulate these processes. Formation of reassortants, as determined by polyacrylamide gel electrophoresis of RNA segments, occurred between all strains of human rotavirus tested. Reassortants that formed between subgroup 1 and 2 strains, however, were replaced within several passages by the parental subgroup 2 viruses. This correlates with the observed lack of reassortants between subgroup 1 and 2 viruses among natural isolates. Coinfection with subgroup 2 strains (3 sets were tested) consistently resulted in the selection of specific reassortant progeny and the rapid disappearance of parental viruses within several passages. Selection of specific RNA segments in progeny rotaviruses depended on the parental strains used in coinfection. This selection was altered both by changes in the relative multiplicities of infection and growth properties of the parental viruses. The mechanisms used to determine segment selection are under investigation.

R11.37

ANTIGENIC CHARACTERIZATION OF ROTAVIRUSES ISOLATED IN KENYA FROM 1982 TO 1983. S.Urasawa,T.Urasawa,Y.Chiba,K.Taniguchi, L.N.Mutanda and P.M.Tukei. Departments of Hygiene and Pediatrics, Sapporo Medical College,Sapporo,Japan,and Medical and Virus Research Centers, KEMRI, Nairobi, Kenya.

Previously, the electropherotype of human rotavirus(HRV) RNA from 100 diarrheic stool specimens collected in two major districts of Kenya in 1982-1983 was reported (J.Clin.Microbiol.19,p579,1984). Of these,25 stool specimens containing HRVs with different electropherotypes were subjected to virus isolation experiment with MA104 cells, and 16 HRV strains were isolated. The use of ELISA with subgroup(sub.)-specific monoclonal antibodies and fluorescent focus neutralization test with serotype(sero.)-specific rabbit sera enabled us to succesfully subgroup 13 isolates(4:sub.I &9:sub.II) and to serotype 13 isolates(7:sero.1,4:sero.2 & 2:sero.3). Of the 3 isolates having the unidentified subgroup antigen, F153 and G402 were equally neutralized by the sero.1 and sero.4 antisera and by the sero.3 and sero.4 antisera,respectively,while F132 was determined as sero.3. In addition, F247 having sub.II antigen was also equally inactivated by both sero.3 and sero.4 antisera. Further characterization of the 3 strains having seemingly dual serotype specificity was carried out using antisera raised against F153 and F247 as well as sero. 1-4-specific VP7-directed monoclonal antibodies. The result clearly indicated that F153 is a serotypic mosaic strain having the sero.4-specific VP3 and the sero.1-specific VP7. Complex antigenic structure of G402 and F247 was also suggested.

R11.38

POTENTIALLY NEW VIRAL STRUCTURE FROM HUMAN DIARRHOEAL SPECIMENS

G. Lecatsas, M.I. Skikne, I.W. Windsor.
Department of Medical Virology, Institute of Pathology, Pretoria; National Institution of Virology, Sandringham; Department of Microbiology, University of Natal, Durban.

Electron microscopic investigations on 21 stool specimens from cases of diarrhoea using the technique of negative staining have yielded virus-like particles with a clearly defined subunit structure. Although the forms are reminiscent of tubular structures encountered in certain rotavirus preparations, no icosahedral structures have been detected in the six positive specimens. Although these structures may represent variants of a rotavirus, they may also represent a new pleomorphic agent which may play a role in human viral diarrhoea.

R11.39

THE GENETIC, BIOLOGIC, AND ANTIGENIC RELATIONSHIPS AMONG AVIAN AND MAMMALIAN GROUP A ROTAVIRUSES.

SM Matsui, RD Shaw, ER Mackow, HB Greenberg. Department of Medicine, Division of Gastroenterology, Stanford University, Stanford, CA, USA.

Rotaviruses (RV) are an important cause of severe diarrhea in the young of many species. Some avian RV share common group antigens with group A mammalian strains.

We studied a series of group A avian and mammalian RV to determine the extent of their relatedness at the protein and nucleic acid levels. All of the structural proteins of avian and mammalian RV were antigenically indistinguishable by Western blot analysis using hyperimmune sera. However, group A avian RV were distinct from mammalian strains in neutralization studies and in solid phase immunoassays using a variety of broadly cross-reactive monoclonal antibodies. When studied by Northern blot analysis under conditions designed to detect ~50% nucleic acid sequence homology, there was no apparent cross-hybridization of mammalian and avian RV nucleic acids.

We found that avian and mammalian RV do not reassort or reassort at a frequency below 0.25% during coinfection of susceptible cells with both strains of RV. This implies a lack of genetic relatedness among avian and mammalian group A RV. Preliminary mRNA sequence data indicate that the first 8 bases at the 3' end are conserved in avian and mammalian RV. The molecular basis for the restriction of gene reassortment is being studied further.

R11.40

DETECTION OF SUBGROUP F ENTERIC ADENOVIRUSES: SPECIFICITY AND SENSITIVITY OF MONOCLONAL ANTIBODY ELISA, RESTRICTION ENDONUCLEASE ANALYSES, HYBRIDOT AND ELECTRON MICROSCOPY.

N. Singh-Naz[1], W. Rodriguez[1], A. Kidd[2], and C. Brandt[1].
Department of Infectious Diseases[1] and Virology[1], Faculty of Children's Hospital National Medical Center[1], National Institute of Virology[2], George Washington University[1], Washington, D.C., U.S.A.[1], Sandringham, South Africa[2].

Subgroup F enteric adenovirus (EAd) specific monoclonal antibodies 5D8/2C2, 2H6/1E11 and 3F11/2H9, which respectively recognize both Ad40 and 41, Ad40 alone and Ad41 alone were used as dector antibodies in an ELISA to study 51 different isolates from patients with EAd. Corresponding stool suspensions were also analyzed for 42 specimens. The ELISA results of the 51 EAd specimens were in concordance with the restriction enzyme analyses pattern(34/34 viruses) and hybridot (17/17 viruses). Monoclones 2H6/1E11 and 3F11/2H9 identified 10 Ad40 and 24 Ad41 viruses in specimens typed by restriction endonucleases analyses. One specimen which was EAd-positive by ELISA both as a fecal suspension and a 293 cell culture isolate was identified as non-EAd by neutralization, was cut by Eco RI or KpnI in a pattern not consistent with Subgroup F EAd, and gave a doubtful result by hybridot. Selected stool specimens were further studied in parallel dilution by ELISA and by electron microscopy to determine the extent to which low titer fecal EAd samples can be accurately assayed. The ELISA detected all four specimens at dilutions where virus was no longer seen by EM.

R11.41

IMMUNIZATION OF SWEDISH CHILDREN WITH THE RHESUS ROTAVIRUS VACCINE (RRV) MMU 18006.

P. Juto[1,2], G. Wadell[1,2], R.I. Glass[3], A.Z. Kapikian[3], L. Gothefors[2,4]. Dept. Virol.[1] Pediat[2] Univ. of Umeå[3] Sweden, NIAID[3],NIH, Bethesda, USA.

RRV reacts in neutralisation tests as human rotavirus 3. Evaluation of acceptance, seroconversion and protection against rotavirus infections during two seasons after vaccination with the RRV vaccine was performed in the following way: Group A-106 children aged 5-12 months were randomised to oral administration of 10^5 $TCID_{50}$ of RRV or placebo and then followed for 18 months. Sera were drawn on day 0; after 1 and 6/18 months and analysed by CF and ELISA. Stool specimens were collected, whenever lose stools were reported, and analysed for presence of rotaviruses, adenoviruses or enteropathogenic bacteria according to Uhnoo et al. J. Infect. 13, 73, 1986. Group B-35 children aged 2-5 months were randomised to 10^5 $TCID_{50}$ of RRV or placebo and followed for six months. Reactions to RRV:Diarrea >1 day: Group A RRV 30%, placebo 10%; Group B RRV 22%, placebo 8%. Fever >38°C: Group A RRV 80%, placebo 8%; Group B 2-3 months old 33%, 4-5 months old 71%, placebo 25%.
Serological responses 4 weeks after vaccination; Group A: RRV CF 86%, Placebo 2%; ELISA IgG and/or IgA RRV 93%, Placebo 4%; Group B ELISA IgG RRV 70%, Placebo 0%.

R11.42

ISOLATION AND CHARACTERIZATION OF A POSSIBLE INTERTYPIC ROTAVIRUS THAT BELONGS TO SUBGROUP I BUT HAS A "LONG" RNA PATTERN TYPICAL OF SUBGROUP II HUMAN ROTAVIRUSES.

O. Nakagomi, T. Nakagomi. Departments of Laboratory Medicine and Microbiology, Akita University School of Medicine, Akita 010, Japan.

From a total of 156 rotavirus specimens that were obtained from Japanese children with acute gastroenteritis during the period between December 1981 and April 1983, we have identified five different RNA electropherotypes (all of them were long patterns) in the 81/82 winter epidemic, whereas all but one that were obtained in the 82/83 epidemic showed an identical RNA patterns. Unexpectedly, one of the 81/82 isolates was identified as a subgroup I rotavirus in spite of possessing a long RNA pattern (faster moving 10th and 11th gene segments upon gel electrophoresis). We have successfully isolated this virus, designated as AU-1, in cell culture and characterized AU-1 by RNA-RNA hybridization in relation to various human and animal rotaviruses. The AU-1 strain is not a simple reassortant between subgroup I and II human rotaviruses but it shares a high level of sequence homology only with the gene encoding VP7 of serotype 3 human rotaviruses. Weak homology was also observed between AU-1 and some other animal rotaviruses suggesting a possible in vivo reassortment between human and animal rotaviruses.

R11.43

Primary Characterization of VP3 neutralization escape mutants. E. R. Mackow, R. D. Shaw, S. M. Matsui, P. T. Vo, H. B. Greenberg.

A series of mutations in the genes coding for the two rhesus rotavirus surface proteins, VP3 and VP7, were selected by growth in the presence of neutralizing monoclonal antibodies. Mutations in VP3 were selected independently by growth in the absence of trypsin. Mutations were produced on both VP3 and VP7 in epitopes specifying either homotypic (serotype 3) or heterotypic (serotypes 1,3,4,5,6) neutralization. The neutralization epitopes on VP7, with one exception, were not detected on western blots while the neutralization epitopes on VP3 appear to be less dependent on protein conformation. The VP3 mutations selected with trypsin deficient media altered plaque morphology and growth. The location of these mutations in the coding sequence of genes 4 and 9 is being determined from the plus stranded messenger RNA using an oligonucleotide sequening strategy.

R11.44

A comparison of the efficiency of seven different rapid diagnostic tests for the detection of Rotavirus in stool specimens from patients at a Children's Hospital.

E. Thomas, E. Kawano, M. Curran, N. Petermann.

We have evaluated the performance of 7 commercially manufactured rotavirus tests (4 ELISA/3 latex). 140 stool specimens were assayed (patient age: 1 month-19 years). Results obtained were compared with electronmicroscopy (EM) results. ELISA tests: (Rotazyme 11, Abbot; Pathfinder, Kallestad; Rota immunoassay, IDL Inc.; Enzygnost Rota assay, Behring). Latex: Meritec-Rota latex test, (Meridian), Virogen Rotatest, (Whampole) and a Rota latextest (Bartel). Test outcomes were compared to EM on the basis of sensitivity, specificity, crossproduct ratio (CR) and to each other (because of the absence of a true "gold standard") on the basis of CR only. (CR=ratio of the odds of test A being pos. when test B is pos. to the odds of test A being pos. when test B is neg. When CR 1, the greater its value, the more similar the tests). Results (EM as "goldstandard"): Sensitivity: Rotazyme 92%, Pathfinder 89%, Bartel 86%, Virogen 86%, Meritec 78% and IDL 75%. Specificity: Virogen 95%, Meritec 92%, IDL 91%, Pathfinder 85%, Bartel 72% and Rotazyme 71%. CR: 116 for Virogen/EM and thus the test "most similar to EM." Bartels/EM CR=16, and thus "least similar to EM." Age and sex of the patients had no influence of outcome of the test. Rapidity/cost/labour efficiency and performance data makes Virogen our preferential choice for rapid/accurate rotavirus diagnosis.

R11.46

ROTAVIRUS CARRIAGE.

[illegible] [illegible].

Department of [illegible], Faculty of Medicine [illegible]

Centre Hospitalier Universitaire [illegible], F [illegible].

To study immunity to rotaviruses, specific faecal and serum rotavirus immunoglobulins (rIg) were assayed and the results compared with clinical and epidemiological data. In a first group of children infection occurred in the absence of both local and systemic rotavirus antibodies and elicited a specific local response of copro rIgA and rIgM in addition to a seroconversion for rIgM, rIgA and rIgG. During such acute infections gastroenteritis could typically appear. The second type of infection, carriage, was observed in children lacking local antibody but protected by serum antibodies, rIgG or rIgA, of maternal or acquired origin. Although diarrhoea was present in [illegible] % of patients of the second type, the clinical pattern resembled that produced by other agents found in the community at the time. In a third group, without infection, there was a high prevalence of local rIgA. In this study, breast feeding was not correlated with protection against infection. These data show that the immune response was either local plus systemic or local only. They indicate that systemic antibodies predict resistance to illness but not to virus colonization, whereas local antibodies predict protection against infection. They also suggest that it is necessary to detect serum rIgM or a rising antibody titre, and not only rotavirus shedding, in order to distinguish true rotavirus-induced diarrhoea from diarrhoea in a rotavirus carrier.

R11.48

SEROEPIDEMIOLOGY OF ROTAVIRUS INFECTIONS IN PREMATURE INFANTS AND CHILDREN. R.I. Glass, H.S. Ho, M. Penaranda, T. Calk, B. Harrison, T. Durham, P. Woods, L. Anderson. Centers for Disease Control, Atlanta, GA., U.S.A.

To examine the possible role of maternal and naturally acquired antibody in altering the response to RV infection of premature and term infants and children, we tested cord blood from newborns and sera from children 0 to 2 years of age for IgG and IgA antibodies to RV.

IgG antibody titers were lowest in premature infants with the lowest birthweights and increased 16 fold in full term newborns. The prevalence declined during the first four months of life and then increased continuously to peak at age 2 years when the prevalence was >80%. IgA antibodies were lowest early in life and rose continuously but to lower titers with age. These data are consistent with transfer of maternal antibody late in pregnancy, its disappearance in the first month of life, and the subsequent appearance of both IgG and IgA antibody from natural infection. They can explain the occurence of more symptomatic RV infections in premature than in term infants, the low rates of symptomatic RV infection in infants in their first months of life, the peak of RV diarrhea from 3-18 months of age, and the subsequent decline in RV diarrhea with increasing age. Premature infants may be at greater risk than term infants for adverse reactions to RV vaccines currently being developed.

R11.45

ANTIGENIC AND POLYPEPTIDE STRUCTURE OF BOVINE ENTERITIC CORONAVIRUS : CHARACTERIZATION OF THE PEPLOMERS PROTEINS. J.F. Vautherot and J. LAPORTE. Station de Recherches de Virologie et d'Immunologie, 78850 Thiverval-Grignon, France.

A panel of 44 monoclonal antibodies was used to characterize the 3 outer glycoproteins (GP) forming the virion peplomers : GP 105, GP 200 and GP 65/125. GP 105 and GP 200 derived from a common precursor P 180 but differed in their sensitivity to limited proteolytic cleavage and by the antigenic determinants they expressed. Cleveland mapping was used to assess their differences and elucidate the maturation process. GP 65/125 was found to be antigenically and structurally different from the two other GP. Neutralizing epitopes were identified on GP 105 and GP 65/125 ; the latter protein displayed hemagglutinating activity (HA) as monoclonal antibodies against it had both neutralizing and HA inhibition activity (HAI). The distribution of neutralizing epitopes on GP 105 and GP 65/125 was studied by selection of mutant viruses resistant to neutralization and competition binding of monoclonal antibodies. Neutralization epitopes mapped in 4 domains on GP 105 and, at least, 3 domains on GP 65/125. GP 65/125 expressed 2 epitopes conserved in hemagglutinating coronaviruses, one of them being recognized by a neutralizing monoclonal antibody. The antigenic and polypeptide structure of BECV peplomers was identical to that reported for OC 43 and DVIM, GP 200 and GP 105 being similar to the E2 complex of MHV while GP 65/125 supported the HA activity and a major part of the virus-cell interaction.

R11.47

EFFECT OF ROTAVIRAL INFECTION AND NUTRITIONAL MANIPULATION ON THE UPTAKE OF MACROMOLECULES IN MICE. I. Uhnoo, J. Freihorst, M. Riepenhoff-Talty, P.L. Ogra. Depts. Pediatrics and Microbiology, Faculty of Medicine, SUNY at Buffalo, Div. Infect. Dis., Children's Hospital, Buffalo, New York, USA.

Groups of malnourished and normally nourished suckling mice were sham infected or inoculated orally with rotavirus at six days of age. Three days post-infection, when viral replication and mucosal damage were established, the animals were given a single dose of 100 µg of ovalbumin (OVA) via an intragastric feeding needle. At different intervals OVA concentrations were determined in serum, gastric content, intestinal lavage fluid and intestinal tissue homogenates, employing the techniques of ELISA and immunofluorescence.

In all mice, OVA was readily absorbed in the gut and could be consistently detected in serum within 5 minutes in concentrations ranging from 17 to 80 ng/mL. OVA levels in serum peaked around 20 minutes after administration and then the activity rapidly declined and little OVA was detected after 60 minutes. Approximately 10-20% of a given OVA dose was absorbed systemically. Significantly, however, the serum level of OVA was higher in rotavirus-infected and malnourished animals. The absorption of OVA tended to be influenced in part by the gastric empyting time and the dose of OVA administered.

The findings suggest that rotavirus infection alters the macromolecule uptake in normally and nutritionally damaged mice.

R11.49

CELL MEDIATED IMMUNE RESPONSES TO ROTAVIRUS IN BREAST MILK AND PERIPHERAL BLOOD. Department of Virology, UMDS of Guy's & St Thomas' Hospitals, London, UK. B M Totterdell, J E Banatvala, I L Chrystie.

Although breast feeding protects against rotavirus infection, this is not mediated by lacteal antibodies. Employing a lymphoproliferative assay, we found that cell mediated immune responses to rotavirus antigen (SA11) was present in the breast milk of lactating mothers as well as in peripheral blood. The response in breast milk did not necessarily correlate with rotavirus IgA antibodies.

Peripheral blood samples were collected from elderly patients with and without rotavirus infection, post renal transplant patients, an immunodeficient child who had been excreting rotavirus for eighteen months, young Finnish adults vaccinated with rotavirus vaccine and healthy laboratory personnel. Following rotavirus infection, the rotavirus induced systemic lymphoproliferative responses peaked at 9-14 days and declined to low levels by 35-40 days. Renal transplant and elderly patients had a significantly lower response than the healthy group ($p=0.02$ and 0.04); no response was detected in the cellular immunodeficient child. The vaccinees, all of whom had pre-existing rotavirus antibodies, had no significant increase in proliferative response, or in specific IgG post vaccination.

Successful vaccination may be dependant on a clearer understanding of the local and systemic rotavirus induced immune responses.

R11.50

DIFFERENT CELL SUBSTRATES SELECT DIFFERENT POLIOVIRUS TYPE 2 VARIANTS FROM CLINICAL SAMPLES. John P.M. Clerx and Herbert Meier-Ewert, Dept. Molec. & Cell. Biol., Univ. of Arizona, Tucson, AZ (USA) and Institute for Medical Microbiology, Technical University Munich, (GFR).

Stool samples from a hypogammaglobulinemic adult patient were found to contain multiple variant viruses as defined by T1-oligonucleotide fingerprinting and reaction with monoclonal antibodies of plaque-purified viruses. One homogenate was inoculated onto 6 different cell substrates and the resultant virus isolates were labeled in vivo. Fingerprints of such viruses indicate that: 1] some cell lines select only one variant from the virus population (HEp-2, BGM), 2] most cell substrates allow the amplification of a variety of virus variants, albeit different variants between different cell substrates (Vero, LLC-MK2), HeLa, Cynomolgus kidney), 3] freezing of virus containing stool homogenates reduced the virus titer and the heterogeneity of variants. The selection of variants is the result of an early stage in infection, since plaqueing efficiency of virus in stool homogenates was similar on the different cell substrates. There was no correlation between permissiveness (amplification of variants) and virus replication time in different cells. At higher multiplicities of infection than in isolation attempts (>0.001 p.f.u/cell), "restrictive" cells faithfully replicated the diversity of input virus.

R11.51

HETERODUPLEX GEL ANALYSIS OF BOVINE ROTAVIRUS STRAINS A.J. Herring, N.F. Inglis, D.R. Snodgrass, M.A. McCrae, Moredun Research Institute, 408 Gilmerton Road, Edinburgh, Scotland, UK., and Department of Biological Sciences, University of Warwick, Coventry, UK.

At least 3 serotypes of bovine rotavirus have been isolated. Experimental studies indicate they do not cross-protect in passive immunisation experiments. Available vaccines usually contain a single serotype 6 strain of rotavirus. Serotype is largely determined by VP7, a product of gene segment 8, and a technique to compare relatedness of this gene from field strains has been developed. This is important to determine optimum vaccine composition.

VP7 gene from the vaccine strain of rotavirus has been subcloned to Gemini transcription vector. A labelled ssRNA copy of this gene is hybridised to test virus dsRNA. The resulting hybrid molecules are digested with ribonuclease so that ssRNA is digested but ds regions are left intact. Analysis of the size of fragments by gel electrophoresis enables the degree of relatedness to be estimated. Results with culture and faecal rotavirus strains of known serotype have validated the technique.

R11.52

ANALYSIS OF ELECTROPHEROTYPES OF ROTAVIRUS DETECTED FROM DIARRHOEIC FAECES OF NEONATAL BUFFALO CALVES IN INDIA, Ajit Singh, R. Pandey, Virel Diarrhoea Research Unit, Department of Veterinary Microbiology, Faculty of Veterinary Medicine, Haryana Agricultural University, Hisar, India

RNA migration profile of rotavirus strains collected from diarrhoeic faeces of neonatal buffalo calves was investigated in an organised buffalo farm employing polyacrylamide gel electrophoresis (PAGE) followed by silver staining. Changes in RNA electropherotypes during the progress of disease were recorded. Simpler but less sensitive tests such as agar gel immunodiffusion (AGID) and discontinuous counterimmune electrophoresis (DCIE) were used as initial screening tests in the course of investigation. Diarrhoeic cases were recorded from Oct., 1986 to Jan., 1987. The two assays AGID and DCIE were found less sensitive than PAGE. Of the faecal samples collected only 23.63% and 21.81% were positive by AGID and DCIE respectively and 36.36% were positive by PAGE.

R11.53

SEQUENCE ANALYSIS OF MUTATIONS IN IMMUNODOMINANT NEUTRALIZATION EPITOPES OF ROTAVIRUS SA-11. M. Gorziglia, K. Taniguchi, Y. Hoshino, R. Jones, K. Nishikawa, R.L. Ward, A.Z. Kapikian and R.M. Chanock. National Institutes of Health, Bethesda, Maryland, U.S.A.

Rotaviruses possess two outer capsid proteins, designated VP7 and VP3, which are considered to be independent neutralization antigens. Replication of rotavirus SA-11 in the presence of hyperimmune antiserum resulted in the emergence of variants which were poorly neutralized by the antiserum. Nucleotide sequence analysis of six variants revealed a total of nine distinct amino acid changes on VP7 and seven on VP3. Amino acid changes were found in six variable and in two conserved regions in VP7. These changes were predominantly located in the glycosylation site and in the major immunodominant antigenic site on VP7 involved in rotavirus neutralization. In VP3, two of the seven regions affected by amino acid changes may represent immunodominant antigenic sites. One of these antigenic sites (amino acid residue 392) bears an epitope that is shared by serotypes 1,3, and 4.

Analysis of the amino acid sequences of rotaviruses belonging to different serotypes and their variants selected either in the presence of polyclonal antiserum or monoclonal antibodies suggests that immunodominant epitopes are located at the same position regardless of serotype specificity.

R11.54

LOCALIZATION OF CROSS-REACTIVE AND SEROTYPE-SPECIFIC NEUTRALIZATION EPITOPES ON HUMAN ROTAVIRUS VP3 AND VP7. K. Taniguchi, M. Gorziglia, Y. Morita[1], T. Urasawa[1], S. Urasawa[1], A.Z. Kapikian, and R.M. Chanock. National Institutes of Health, Bethesda, Md., U.S.A.; 1. Dept. of Hygiene, Sapporo Medical College, Sapporo, Japan.

Two outer capsid proteins of rotavirus, VP3 and VP7, bear neutralization epitopes. In tests employing 7 VP3 cross-reactive neutralizing monoclonal antibodies (N-mAbs) against human rotavirus (HRV) serotype 1,3, or 4, we observed some intraserotypic antigenic variation as well as marked differences in reactivity patterns between serotype 2 strains and serotype 1, 3 and 4 strains. In contrast, 9 VP7 neutralizing monoclonal antibodies were serotype specific. Epitope analysis of antigenic variants of serotype 1, KU strain, resistant to N-mAbs showed that VP3 has at least 4 apparent independent cross-reactive neutralization epitopes, while VP7 has at least 5 apparent overlapping serotype-specific neutralization epitopes which contribute to a single antigenic site.

Nucleotide sequencing of the antigenic variants by primer extension permitted us to identify the nucleotide substitutions in the VP3 and VP7 genes of the antigenic variants. Amino acid changes were detected at residues 305, 392, 433 and 439 of VP3 and at 94, 97, 211 and 213 of VP7. These data may prove useful in current efforts to develop vaccines against HRV infection.

R11.55

MOLECULAR EPIDEMIOLOGY OF HUMAN ROTAVIRUSES. K. Green, J. Sears, Dorys Garcia[1], K. Midthun, M. Gorziglia, Y. Hoshino, R.M. Chanock, A.Z. Kapikian, and J. Flores. National Institutes of Health, Bethesda, Maryland, U.S.A.; 1. Instituto de Biomedicina, Caracas, Venezuela.

Sequence analysis of the RNA segment encoding VP7 (the major neutralization protein) from nine different rotaviruses belonging to four serotypes revealed a high degree of conservation in this gene among strains that share the same serotype specificity regardless of geographical origin or time of isolation. Conversely 6 major regions of divergent sequence were detected among strains belonging to different serotypes. These relationships enabled us to develop: (a) a rapid nucleotide sequencing procedure and (b) a dot hybridization assay using specific cDNA probes corresponding to divergent regions of the VP7 gene both of which correctly predicted serotype specificity. Both procedures bypass the need for tissue culture isolation and propagation of the viruses. A similar approach has been employed to detect specific sequences of the VP3 gene that appear to be involved in attenuation of rotaviruses that cause asymptomatic infection in neonates and that are capable of persisting in newborn nurseries over an extended interval.

R11.56

DETERMINING THE ROTAVIRUS SUBGROUP WITH MONOCLONAL ANTIBODIES BY ELISA. V. Yonkova. Department of Microbiology & Virology, Higher Institute of Medicine, 9002 Varna, Bulgaria.

For the rapid and accurate identification of Rotavirus in faecal specimens of patients with acute gastroenteritis we used a kit from WHO with monoclonal antibodies for ELISA.

112 specimens were investigated and 24(21 %) of them were positive for Rotavirus subgroup I, 71(63 %) - for subgroup II and 17(16 %) could not be grouped.

Comparative studies of the investigation of faecal specimens for the detecting of Rotavirus by other methods correlate well.

R11.57

ORALLY ADMINISTERED REASSORTANT PORCINE ROTAVIRUS PREVENTS DIARRHEA IN GNOTOBIOTIC PIGLETS UPON CHALLENGE WITH VIRULENT PARENTAL PORCINE ROTAVIRUSES

Y. Hoshino, L.J. Saif*, M.M. Sereno, R.M. Chanock, and A.Z. Kapikian. Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, and *Ohio State University, Wooster, Ohio, U.S.A.

A single gene substitution OSU x Gottfried porcine rotavirus reassortant was generated. This reassortant derived 10 genes from OSU strain(serotype 5) and only VP7 gene from Gottfried strain(serotype 4). Sera obtained from gnotobiotic piglets three weeks after initial oral administration of this reassortant (VP3:5; VP7:4) contained high levels of neutralizing antibodies not only to Gottfried VP7 but also to OSU VP3. When these animals were challenged orally three weeks later with virulent Gottfried virus (VP3:4; VP7:4), complete protection was observed as judged by failure of symptoms to develop and lack of virus shedding. Following challenge with virulent OSU virus(VP3:5; VP7:5), almost complete protection against diarrhea was also observed and virus shedding was delayed in onset, was decreased in duration. The relevance of these findings to the development of effective reassortant rotavirus vaccines will be discussed.

R11.58

IMMUNODOMINANT NEUTRALIZATION EPITOPES OF VP7 OF SIMIAN ROTAVIRUS SA11. G.Gerna, A. Di Matteo, A. Sarasini, M. Battaglia. Virus Lab, Institute of Infectious Diseases, University of Pavia, 27100 Pavia, Italy.

Two VP7 immunodominant neutralization (Nt) epitopes of simian rotavirus SA11 were identified by highly Nt monoclonal antibodies (MAbs) in competitive binding assays. Group A MAbs competed partially with group B members, whereas group B MAbs totally competed with group A members. In addition, the two epitopes were identified by differential HI activity of Nt MAbs. Finally, a VP6-reactive MAb slightly competing , to a different degree, with Nt MAbs to the two epitopes, was differentially enhanced in its binding by Nt MAbs of the two groups. Studies of immunodominant epitopes of VP7 (and VP3) of different HRV serotypes could help in the preparation of a synthetic or DNA recombinant vaccine. Partially supported by WHO contract HQ/86/075264 and by Regione Lombardia, Ric. Finalizzata, Project No. 875.

R12.1

CITRUS EXOCORTIS VIROID DETECTION AND SOME BIOLOGICAL PROPERTY ANALYSIS BY DOT-BLOT HYBRIDIZATION
Z.Yongzhi, C.Yali, G.Zongming, D.Sujun, D.Daming, Wuhan Institute of Virology, Academia Sinica, Wuhan China. Nucleic acid extracts from exocortis citrus leaves and the seed, root, stem and leaves of healthy-looking citrus and CEV-infected symptom and symptomless C.aurantiaca leaves were analysed by dot-blot hybridization using a probe of 20 bases, which was automatically synthesized in a DNA synthesizer according to the sequence complementary to the conserved central region of 7 viroids. The results show that the probe is high specific and CEV exists in seed, root, stem and leaf of citrus. Different amount of CEV was found in exocortic and latent CEV-infected citrus, which is considered that exocortic symptom expression is related to CEV concentration in citrus. Sample was denaturated by boiling and formalamine treatment before being spotted onto nitrocelliulose membrane to break base-pairing structure in CEV. The technique lead to detection of 3-4 ng CEV in 0.4g of fresh leaves. More than a hounared samples could be checked in a week. It has been successfully applied to detect CEV and PSTV in field and nursery samples and, theoretically, it can be used to detect 7 viroids.

R12.2

SMALL COVALENTLY CLOSED CIRCULAR RNA IN PLANT.
D.Daming, D.Sujun. Wuhan Institute of Virology, Academia Sinica, Wuhan, China

PAGE of nucleic acids from privet (Ligustrum compactum) revealed the pregence of RNA with mol. wt. of approximately 0.1×10^6. The RNA was found to be in covalently closed circular form by means of labeling analysis, two-dimensional and bidirectional PAGE followed by silver staining. Highly intramolecular base-pairing structure was detected in the RNA. Most reported circular viroid RNAs have a conserved central region but circular RNA from privet lacks such a structure according to the dot-blot hybridization result using a synthesized deoxyoligonucleotide probe. No viral particles were observed in the sap and solutions prepared according to the procedure designed to isolate viruses. The RNA was not transmissible to several indicator plants. Such kind of circular RNA was also detected in 2 other kinds of plant. No strains of the above 3 kinds of plant without small circular RNA have been found and also no symptom related to small circular RNA has been observed. It is suggested that the covalently closed circular RNA in higher plants may be a relatively popular phenomenon and they may be naturally exist in the plants, so it's very important to distinguish them from circular viroids.

R12.3

USE OF A CLONED NON-STRUCTURAL GENE FOR THE DETECTION OF BROMOVIRUSES. E.P. Rybicki, M.B. von Wechmar, M.I. Parker*. Depts. of Microbiology and Medical Biochemistry*, University of Cape Town, South Africa.

The Bromovirus group of plant pathogens has six putative members. Brome mosaic virus (BMV, type member) has a 3-segment ssRNA genome: RNAs 1 and 2 are monocistronic; RNA 3 (2114 nt) codes for a 5'-proximal 3a protein gene and a capsid protein (cp) gene. There is considerable intra- and interviral homology between the ± 160 3'-terminal bases of all RNAs; consequently, cDNA probes made by 3'-priming might not be very specific. We chose to construct a BMV 3a gene probe to avoid this problem, and because 3a gene sequences are more homologous than cp sequences. cDNA transcription of BMV RNA 3 was primed by binding oligo dT to the intergenic poly A sequence. dsDNA was dC-tailed, annealed with dG-tailed PstI-cut pBR322, and cloned in E. coli. The largest recombinant (pB3a2) was mapped and used in hybridisation tests with Bromovirus RNAs. The specificity of the probe, and the relative merits of ^{32}P and biotin labelling, are discussed.

R12.4

BI-DIRECTIONAL ELECTROPHORESIS, A GOOD ALTERNATIVE FOR VIROID DETECTION USING NUCLEIC ACID PROBES. H. Huttinga and W.H.M. Mosch, Research Institute for Plant Protection, Wageningen, the Netherlands.

Bi-directional electrophoresis (BDE) in combination with a silver-nitrate staining is a sensitive and quick method to detect circular RNAs which have a high degree of internal base-pairing like viroids and viroid-like RNAs. An improved version of the original method does not require the handling of gel pieces from the native gel to the denaturing gel. After the first run denaturing conditions are created in the native gel by increasing the temperature of the electrophoresis buffer and the gel to 75 °C. The second run under denaturing conditions can thus be made in the same gel by just reversing the polarity of the electric field.

The simplified method was compared with a molecular hybridization method using ^{32}P as a marker system for its use in detecting potato spindle tuber viroid and chrysanthemum stunt viroid. The results indicated that BDE is as sensitive as is the molecular hybridization test. It can detect viroid in leaves at concentrations as low as 10 ng/g of leaf. This means that one diseased leaf among 499 healthy leaves can be detected. The method does not require hazardous chemicals like ^{32}P and formamide. It is also more reliable than molecular hybridization because it is based on two criteria: a staining reaction, situated at a specific site.

R12.5

DETECTION AND CHARACTERIZATION OF VIRUSES IN ORNAMENTAL BULBS USING cDNA CLONES
H.J.M. Linthorst[1], C.I.M. van der Vlugt[2], C.J. Asjes[3] and J.F. Bol[1]. [1]Department of Biochemistry, State University of Leiden; [2]Dutch Bulb Inspection Service and [3]Bulb Research Centre, Lisse, The Netherlands.

To initiate a study on the possibility of using cDNA for the detection of viruses in ornamental bulbs, we have cloned different tobacco rattle virus (TRV) strains. Characterization and nucleotide sequense of TRV strains PSG and TCM has been published (N.A.R. 14, 2157-2169 and 4673-4682). RNA 1-derived clones were capable of detecting TRV infections in tulip and narcissus. Clones derived from RNA 2 of the two strains hybridized with different subsets of TRV. Hybridization of a TCM-RNA 2 clone with a pea early-browning virus (PEBV) isolate, parallel to ELISA results showing a serological relationship between TCM and PEBV prompted us to characterize the PEBV-RNA 2 genome segment. Furthermore, we are presently cloning potyviruses from tulip and narcissus for characterization and detection purposes.

R12.6

A SYNTHETIC OLIGONUCLEOTIDE PROBE DIFFERENCIATES HOP STUNT VIROID AND ITS THREE ISOLATES. T. SANO, H. KUDO, T. SUGIMOTO*, E. SHIKATA. Department of Botany, Faculty of Agriculture, Hokkaido University, Sapporo 060, Japan. *YUKI GOSEI KOGYO Co.,Ltd., Tokyo 174, Japan.

A hop stunt viroid group (HSV group) consists of hop stunt viroid (HSV) from hop and three isolates from cucumber (HSV-c; cucumber pale fruit viroid), grapevine (HSV-g) and citrus (HSV-cit). These four isolates are indistinguishable by their host ranges and pathogenic properties, although the differences in 1 - 16 nucleotide sequences among the isolates have been shown.

We have tried to differentiate these four isolates by hybridization method using a synthetic oligonucleotide probe. It was designed to be complementary to No.51 - 65 of the nucleotide sequence of HSV-g, where 1 and 2 base mismatchings existed in HSV and HSV-c sequences, respectively. The 5' end of it was labelled with ^{32}P, and used for hybridization. When hybridization was performed at 31°C, strong signals of autoradiograph were recognized in both HSV-g and HSV, but only a weak signal was observed in HSV-c. Moreover, when they were hybridized at 41°C, only HSV-g gave the strong signal, while both HSV and HSV-c did not. These results indicate that the synthetic oligonucleotide probe is useful to differenciate the HSV isolates.

R12.7

STUDIES OF SWEDISH BYDV ISOLATES USING CLONED cDNA PROBES.

M. Eweida & P. Oxelfelt, Dept. of plant and forest protection, Swedish University of Agricultural Sciences, Uppsala, Sweden.

Cloned cDNA has been produced to an isolate of barley yellow dwarf virus (BYDV) specifically transmitted by Sitobion avenae (Sa). Serologically this isolate is very closely related to the American MAV isolate. One of the clones is approximately 1400 bp in length. It reacts strongly with its homologous RNA and RNA from other Sa-specific isolates but only weakly or not detectably with isolates transmitted by both Sa and Rhopalosiphum padi. It also hybridises with an Australian cDNA clone specific for PAV-type isolates but not with a clone specific for RPV isolates (clones pBY 82 and pBY 412, Waterhouse et al., J. gen. Virol. 67, 1273 (1986). cDNA to a Swedish isolate of the PAV type is being prepared.

R12.8

INDIGENOUS DOUBLE-STRANDED RIBONUCLEIC ACID DETECTED IN SOME CUCURBITACEAE, SOLANACEAE, AND CHENOPODIACEAE. J. A. Dodds, R. A. Valverde, S. T. Nameth, O. Abdalla, A. Al-Musa, P. R. Desjardins. Department of Plant Pathology, University of California, Riverside, CA 92521, USA

We have detected several dsRNAs in some non-virus inoculated Cucurbitaceae, Solanaceae and Chenopodiaceae. A particular linear dsRNA (MWt = 9.0 x 10^6) which was present in several cultivars of C. annuum was chosen for further characterization. It was distributed in all plant tissues and was not transmissible by dodder, grafting, or mechanical inoculation to dsRNA negative Capsicum spp. Most of the dsRNA in leaf extracts was associated with the chloroplast fraction of leaf extracts. Partially hydrolyzed dsRNA was used for the synthesis of ^{32}P labelled cDNA. In northern blotting, the probe showed homology to the 9.0 x 10^6 dsRNA in numerous cultivars of Capsicum spp., but had no apparent homology with any host DNAs or ssRNAs, dsRNAs of different size detected in Capsicum spp, or dsRNAs of identical size detected in Phaseolus vulgaris and Cucumis melo. There was no obvious correlation between the presence of the 9.0 x 10^6 dsRNA and cultivar phenotype.

The large size, lack of an association with a virus-like particle, and non-transmissibility of this dsRNA indicates that it may not have anything in common with cryptic viruses or graft transmissible viruses.

R12.9

Molecular Cloning of dsRNA Isolated from Virus Infected Plants.

J. Dale, M. Bateson. Plant Pathology Branch, QDPI, Agricultural Research Laboratories, Indooroopilly, Qld. 4068, Australia.

Double-stranded RNA (dsRNA) can be readily extracted from most plants infected with RNA plant viruses. This dsRNA is assumed to represent the replicative form of the infecting virus. The methods for extracting dsRNA from infected tissue are simple, rapid and applicable to virtually all host/virus combinations tested. Thus it is possible to isolate useable quantities of virus-specific RNA without first purifying virions.

The dsRNA of cucumber mosaic virus (CMV) and sugarcane Fiji disease virus (FDV) was separated from DNA and ssRNA by cellulose chromatography, with remaining trace contamination removed by nuclease digestion. The dsRNA was denaturated by boiling in the presence of random primers. From this random primed RNA, dscDNA was synthesized and ligated in the plasmid PUC 12. Clones were selected which specifically hybridized with dsRNA from CMV or FDV infected plants. Some clones were further characterized as to which CMV or FDV RNA segment they hybridized. These clones were then used for dot blot assay on nylon following labelling with biotin.

These methods are currently being used to investigate the dsRNA associated with banana bunchy top disease and other dsRNAs from tropical fruit trees.

R12.10

THE 3'-REGION OF PLUM POX VIRUS RNA : SEQUENCE OF THE COAT PROTEIN GENE AND USE OF CLONED PROBES FOR VIRUS DETECTION IN INFECTED PLANTS. M. Ravelonandro, C. Varveri, D.Gonsalves* and J. Dunez. INRA, Pathologie végétale, BP 131, 33140 Pont de la Maye, France, and *Cornell University, Geneva, New-York 14456, USA.

Plum pox virus (PPV) is a potyvirus with a single-stranded, positive sense RNA of 3.5 10^6 daltons. PPV causes severe damage to millions of stone fruit trees in several countries of Europe and in the mediterranean area. cDNA of PPV RNA was obtained using the Heidecker and Messing's method with pUC9-dT, and the viral specific cDNA inserts were cloned in E.coli JM 103. Molecular hybridization using cDNA clones as probes was used to detect PPV in infected plants. Comparative tests showed that molecular hybridization was more sensitive than ELISA for detecting PPV in infected plants. As little as 1 pg PPV RNA was detected. cDNA clones corresponding to the 3'-terminal region of PPV RNA were sequenced by the Maxam and Gilbert's method and by dideoxynucleotide sequencing with M13 bacteriophage vectors. The coat protein gene consisted of 801 nucleotides and was adjacent to the untranslated 3'-region (215 nucleotides) of PPV RNA and those of other potyviruses (tobacco etch, pepper mottle, tobacco vein mottling, potato virus Y). However, nucleotide sequence analysis of the coat protein gene of PPV revealed considerable homology with the coat protein gene of these potyviruses. Only the N-terminal amino acids regions did not show homology.

R12.11

INFECTIVITY OF RECOMBINANT PLASMIDS CONTAINING NATURAL OR CHIMAERIC VIROID INSERTS. T. Candresse*, T. Diener**, D.Smith** and J. Dunez*. *INRA, Pathologie végétale, BP 131, 33140 Pont de la Maye, France, ** Microbiology and Plant Pathology Laboratory, BARC, USDA-ARS Beltsville, 20705 Maryland, USA.

Full length infectious clones have been obtained for the type strain and for an indonesian strain of tomato apical stunt viroid (TASV). Conditions for infectivity of plasmids containing monomeric inserts have been analyzed and shown to be similar to those observed for potato spindle tuber viroid (PSTV) or citrus exocortis viroid (CEV). Infectious recombinants between the two strains of TASV have been obtained. We have also constructed a set of chimaeric viroid clones using TASV, CEV and chrysanthemum stunt viroid (CSV) as parents. So far, none of the constructs including CSV has proven infectious, possibly because of disruption of the structure of secondary hairpin I. TASV/CEV hybrid constructs are infectious in tomato, a host for the two parents. They are being tested on citrus and gynura plants, hosts for only the CEV parent. The severity of the symptoms observed confirm the influence of the so called "pathogenesis modulating" region but show that its effects are probably modulated by other parts of the molecule.

The non infectious CSV-containing chimeras are currently being introduced in the genome of tomato using Agrobacterium rhizogenes derived vectors, to see if any protection against viroid infection can be obtained by the expression of these molecules in the transformed plant.

R12.12

COMBINATION OF RNA PROBES, TEMPERATURE-GRADIENT GEL ELECTROPHORESIS AND THERMODYNAMICS FOR DETECTION OF VIROID STRAINS OF DIFFERENT PATHOGENICITY

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Hybridization with RNA probes was combined with physicochemical techniques probing the stability of heteroduplexes in order to analyse strains of different pathogenicity of the potato spindle tuber viroid (PSTV). RNA probes were synthesized from clones of several PSTV strains using the T7 polymerase transcription system. As model studies heteroduplexes of (+) and (-) RNA strands from the same and from different clones were produced and analysed by temperature-gradient gel electrophoresis, optical melting curves and thermodynamic calculations. The method of temperature-gradient gel electrophoresis is described in some detail. Base exchanges could be detected and assigned to three separated segments of the molecule corresponding to three resolvable stability regions. Similar studies have been carried out with natural viroids in order to test for and to characterize sequence inhomogenities in crude RNA extracts from infected plants.

R12.13

EVALUATION OF DOT-HYBRIDIZATION ASSAY USING A [^{32}P] RNA PROBE FOR THE DETECTION OF POTATO SPINDLE TUBER VIROID (PSTV). D.K. Lakshman, C. Hiruki, G. Figueiredo, Department of Plant Science, University of Alberta, Edmonton, Alberta T6G 2P5, Canada.

The use of a cRNA probe produced by transcription of pDX4 plasmid by SP6 RNA polymerase in dot-hybridization assay was much more sensitive than the use of a nick-translated cDNA probe from pAV401 plasmic for the detection of PSTV in clarified plant say. This method permitted the detection of 1.4 pg of PSTV mixed in clarified sap from uninoculated leaves. In comparative assays of PSTV-infected potato plants grown in growth chambers at 10, 15, 18, 22, 25, and 30C with light period of 18 hr at a light intensity of 5380 lux, assays with the RNA probe yielded higher signal intensity and higher signal to noise ratios than the DNA probe. When samples from potato plants grown at 25C were used, the assay at 25C yielded the highest signal intensity. Under the optimal conditions, PSTV was detected in a 1/512 dilution of clarified infected plant sap with the RNA probe and a 1/64 dilution with the DNA probe. The autoradiographic spots at a 1/512 dilution with the RNA probe remained prominent as exposure time was extended without background being significantly affected. This was not the case with the DNA probe.

R13.1

CHARACTERIZATION AND COMPARISON OF LACTIC STREPTOCOCCAL BACTERIOPHAGES. J.A. Coveney, G.F. Fitzgerald and C. Daly. Dept. of Microbiology, University College, Cork, Ireland.

Bacteriophages, uc1001 and uc1002, lytic for Streptococcus cremoris UC501 and UC502, respectively, were characterized in detail. Comparisons were made with a previously studied phage, P008, lytic for S. lactis subsp. diacetylactis F7/2 and uc3001 a lytic phage for S. cremoris UC503. Phages uc1001 and uc1002 had isometric heads and non-contractile tails, 30.1 ± 0.6 kb of DNA and cross-complementary cohesive ends. Restriction maps showed no common fragments but there was a very high level of homolgy (83% by heteroduplex analysis) between uc1001 and uc1002 and the organization of both phage genomes was similar. The SDS-PAGE structural protein profiles were related but not identical. Except for restriction maps, P008 was very similar to uc1001 and uc1002. The three showed a very significant bias against the recognition sites of nine out of eleven restriction enzymes. Phage uc3001 was unrelated, in that it had a prolate head and a shorter tail, contained approximately 22 kb of DNA, had unrelated cohesive ends, showed no DNA homology with the isometric-headed phages and displayed a very different structural protein profile. These techniques are being applied to additional phages including representatives from other studies to determine lactic streptococcal phage relationships on a broad basis.

R13.2

IMPORTANCE OF BACTERIOPHAGES IN FERMENTATION PROCESSES. L. Wünsche. Acad. Sci. G.D.R., Institute of Biotechnology, Leipsig, German Democratic Republic

Because any bacterial strain can be infected by virulent phages or carry prophages it must be expected that any technical fermentation process with bacteria is endangered by bacteriophages. A phage development is indicated by serious process disturbances and damages (delayed growth, low and untimely decreasing biomass concentration caused by cell lysis in batch processes; in continuous fermentations a steady state cannot be maintained; the formation of products is diminished or absent; components of mixed cultures disappear; processing of culture suspensions becomes more difficult).

Five methods are available for minimizing the process risk caused by virulent phages, which may be used separately or in combination (phage protected fermentation, use of alternative strains or phage resistent mutants, application of phage inhibitors, and employment of immobilized cells). To prevent damages caused by the induction of the lytic cycle of temperate phages the elimination of inducing factors in fermentation processes and the selection or genetic construction of heavily inducible production strains should be discussed.

R13.3

CORRELATION AND INTERFERENCE OBSERVED BETWEEN PHAGES AND POCK-FORMING ELEMENT IN THIOSTREPTON-PRODUCING STREPTOMYCES AZUREUS. S. Ogata, H. Suenaga, Y. Miyoshi and S. Hayashida. Laboratory of Applied Microbiology, Department of Agricultural Chemistry, Kyushu University, Fukuoka City, 812, Japan.

A wild-type strain (PK0) of S. azureus harbors a pock-forming plasmid pSA1 (8.8 kb; 0.5-1 copy per chromosome) together with chromosomal integrated sequence ($pSA1^{int}$) and also a defective prophage SAt2. The slant or plate cultures of PK0 often show spontaneously developing plaque-like pocks. This appearance brings on an abnormal growth of cultures and a diminished production of thiostrepton. In the pocks, many SAt2 particles were found. However, no pock formation with SAt2 particles was observed in plasmid-free derivatives such as $pSA1^-$ strain (PK100C) and $pSA1^{int^c}$ strain (PKC), and also in other derivatives carrying only autonomous state of pSA1 or its high copy mutant. The revertants from PKC formed the pocks with SAt2 particles. The excision of pSA1 sequence from the chromosome appears to induce the production of SAt2 particles simultaneously. The formation of pocks with SAt2 particles was also inhibited in the PK0(SAt1) which was lysogenized by a temperate phage SAt1 isolated from soil.

Autonomous state of pSA1 or its mutant elicits the different pocks (zones of zygotic inhibition) having no SAt2 particles on plasmid-free strains (recipients) through mating. The formation of these pocks was depressed when the SAt1-lysogenized strain PK100C(SAt1) was used as recipients.

R13.4

ISOLATION AND CHARACTERIZATION OF VIRULENT BACTERIOPHAGES OF STREPTOCOCCUS SALIVARIUS SUBSP. THERMOPHILUS

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During recent years, slow and faulty productions of yoghurt, quarg and cheese due to bacteriophage attacks on thermophilic lactic acid streptococci (S. salivarius subsp. thermophilus) were reported from the dairy industry. For this reason, more than 50 virulent bacteriophages active against S. salivarius subsp. thermophilus were collected from various yoghurt and Swiss type cheese samples from several European countries (Switzerland, France, Federal Republic of Germany).

More than 70 S. salivarius subsp. thermophilus-strains derived from these 3 countries were challenged with these phages and differentiated according to their host pattern: In general, phages from the different countries revealed distinct host ranges and - except for a few phage/host-systems - were unable to replicate on the host bacteria isolated in other countries. The morphology of phages was studied by electron microscopy: All phages examined so far belong to Bradley's morphological group B. They revealed isometric heads (45-60 nm) and long, non-contractile tails of various length (240-270 nm) and thickness (9-13 nm) with small base plates. Collar-like structures were not detected. Sometimes, a single long and thin tail fiber was observed.

R13.5

ENZYMATIC ANALYSIS OF DNAS FROM PHAGE-LIKE PARTICLES AND PLASMIDS OF BREVIBACTERIUM SPECIES. F. Kato, S. Araki, K. Matsuyama and A. Murata. Department of Agricultural Chemistry, Saga University, Saga, Japan.

Phage-like particles are observed by an electron microscope in the lysates of 6 strains that are Brevibacterium ammoniagenes, B. citreum, B. divaricatum, B. imperiale, B. insectiphilium and B. protophormiae. Among them, B. citreum, B. divaricatum and B. protophormiae, carry a plasmid DNA in the cell. Although 6 phage-like particles contain DNA, there is no indicator strain for the particles.

In order to use these DNAs as cloning vehicles in amino acid or nucleic acid producing bacteria system, we analyzed the DNAs with various restriction endonucleases. Restriction patterns of phage-like particle DNAs showed that DNAs were different from each other. And the plasmid DNA of B. citreum had the same restriction pattern of B. divaricatum but different from that of B. protophormiae.

R13.6

INFECTION MECHANISM OF NOCARDIOPHAGE NJL TO NOCARDIA CORALLINA CP222. M. Nakajima and H. Murooka. Department of Agricultural Chemistry, College of Agriculture and Veterinary Medicine, Nihon University, Tokyo, Japan.

Infectivity of nocardiophage NJL to N. corallina CP222 depends strongly on the culture conditions of the host cells. We have already reported that the host cells cultured in a medium containing penicillin markedly decreased the phage infectivity. The causative for this phenomenon appeared to be due to an inhibiting substance for phage infection produced within the host cells cultured in the presence of penicillin, and then, it was demonstrated that lipids, especially phosphatidylethanolamine(PE) was abnormally accumulated within the host cells. The accumulated PE was shown to be responsible for the inhibition of phage infection.

In the present study, we investigated the relationship between quantity of the accumulated lipids in the host cells and electric charge on its cell surface in the infection mechanism of nocardiophage NJL to N. corallina CP222. The phage did not required divalent cations in its infection to the host cells which were cultured without penicillin, whereas the phage required the cations in the infection to the host cells which were cultured with penicillin. The results show that infectivity of the phage is clearly related to the electric charge on the surfaces of the host cells.

R13.7

Bradyrhizobium japonicum phages : Morphology and partial characterization. M. Werquin (1), C. Defives (1), S. Bourey (1) and H.W. Ackermann (2).

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Several phages, specific for Bradyrhizobium japonicum were isolated from soil seeded firstly with soybean. Phages exhibited similar morphologies, showing an icosaedral head of 60 nm in diameter with the presence of spike at the apex. The short tail (14 x 8 nm) showed a baseplate. Chloroform and toluene phage sensitivity suggested the presence of lipids in the capsid. Phages were stable in a 6 to 9 pH range and were heat sensitive for temperatures higher than 65° C.

A temperate phage spontaneously produced by a Bradyrhizobium japonicum strain used as seeds inoculum was also obtained. Phage head was icosaedral with a diameter of 80 nm ; tail dimensions were 109 nm in lenght and 12 nm in width. This data suggested that soil phages were originated from lysogenic bacteria used as soybean inoculum.

R13.8

BACTERIOPHAGES IN THE COLUMBIAN GREEN COFFEE DURING PROCESSING.

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Columbian green coffee is subjected to a fermentation prior to drying. The objective of this artisanal, uncontrolled fermentation is to remove the mucilage surrounding the coffee grains to facilitate subsequent drying. Several types of microorganisms are involved in the fermentation.

In each of the 20 samples of fermenting green bean analysed, bacteriophage specific to certain groups of bacteria were isolated.

Bacteriophages isolated were of the morphological groups Styloviridae and Myoviridae. This is the first report of the presence of bacteriophage in fermenting coffee and illustrates that where one finds large numbers of bacteria one will also find bacteriophage.

R13.9

ϕ KISR 1 - A NEW BACTERIOPHAGE OF OBLIGATE METHYLOTROPHIC BACTERIA.

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The bacteriophage ϕ KISR 1 was isolated from both batch and continuous mixed culture fermentation processes for the production of single cell protein with obligate methanol utilizing bacterial strains.

Using different methylotrophic reference strains the host range of the bacteriophage was found limited to methylotrophic strains KISRI-5 and KISRI-5112 that are related to the genus Methylophilus. The morphology of ϕ KISR 1 differs from these of other known phages which infect methanol utilizing bacteria. ϕ KISR 1 contains double-stranded DNA and is being tested for its application in the development of a host/vector system for methylotrophic bacteria.

R14.1

EFFECT OF VIRAL INFECTION ON Fc RECEPTORS /FcRs/ OF BLOOD POLYMORPHONUCLEAR LEUKOCYTES /PMNs/ DURING MIGRATION IN VITRO. M.Niemiałtowski. Department of Microbiology, Faculty of Veterinary Medicine, Warsaw Agricultural University, 03-849 Warsaw, Poland.

The effect of the migration of bovine blood PMNs in vitro through collagen and monolayer of cells infected by RNA /rotavirus, picornavirus/ and DNA /BHV-1, EHV-1/ viruses on their phagocytic activity was studied. PMNs were examined before and after migration for /i/ EA rosette formation, /ii/ phagocytic activity mediated through FcRs, /iii/ phagocytic activity mediated through non-immunological receptors.

Random migration of PMNs through the infected monolayer to RPMI-1640 with and without chemotactic agent /zymosan activated serum/ and control agents /LPS or fMetLeuPhe/ decreased the percentage of rosettes forming PMNs and phagocytosis mediated through FcRs and nonimmunological receptors.

Results obtained in this study suggest that viral infection could seriously affect cell-mediated immunity and increase the risk of bacterial superinfection.

R14.2

STUDIES ON ANTIBODY SUPPRESSION BY INFLUENZA VIRUS SUBUNIT VACCINE. R. Jennings, T. Smith, R. Whiteside. Department of Virology, University of Sheffield, Sheffield, England.

In vivo experiments in hamsters and mice, and in vitro experiments, using cultures of mouse spleen cells, have shown that preparations containing primarily the surface haemagglutinin and neuraminidase proteins of influenza A(H3N2) or B viruses can suppress the serum antibody response to whole influenza virus vaccine. This effect can be demonstrated using surface influenza virus protein preparations obtained following treatment of whole virus with non-ionic detergent Triton-X-100, or the zwitterionic detergent, Empigen BB, but not with the cationic detergent cetly trimethyl ammonium bromide.

In a series of experiments, suppression of the antibody levels elicited by inactivated whole influenza virus vaccine alone, could be demonstrated, using influenza surface antigen preparations, to occur in both primed and unprimed mouse spleen cell cultures; to be non-specific; to be dependent on the time of addition of the surface antigen preparation relative to that of whole virus vaccine, and to be transferable.

These studies suggest that one reason for the poor immune responses to subunit influenza virus vaccines may be due to a hitherto enrecognised immunosuppressive property of these preparations.

R14.3

GENETIC CONTROL OF THE B CELL AND T CELL RESPONSE TO A SYNTHETIC PEPTIDE OF INFLUENZA VIRUS HEMAGGLUTININ. J.M. Murray, E.M. Anders, X.-L. Yang, D.C. Jackson, D.O. White and L.E. Brown.
Dept. of Microbiology, University of Melbourne, Australia.

Genetic control of the B and T cell response to a synthetic peptide corresponding to the C-terminal 24 amino acids of the HA1 chain of influenza virus hemagglutinin was examined. Inbred mouse strains of the H-2^d haplotype produced high titers of antibody to the peptide and strains of mice of the H-2^b, H-2^k and H-2^q haplotypes gave a low antibody response. Studies with recombinant strains of mice showed high responsiveness to be linked to I-E^d. Antisera from high responder strains of mice were also able to bind to intact virus and this binding was enhanced if the virions had been exposed to pH5, suggesting that acid treatment exposes the C-terminus of HA1. Using synthetic analogues of the native sequence, it was shown that the antibody response is directed predominantly to an epitope in the center of the peptide. A small amount of antibody is directed to a different epitope near the C-terminus. In in vitro T cell proliferation assays it was shown that T cells from peptide-primed mice of the H-2^d haplotype gave a strong response to the peptide while those from H-2^b mice failed to respond. T cells from H-2^k mice could also be stimulated by the peptide despite the poor antibody response of these animals. This may indicate the operation of a suppressor mechanism in the anti-peptide response of H-2^k mice.

R14.4

SHORT LIVED AND WEAK T Cell MEMORY IN CONTRAST TO LONG AND STRONG B CELL MEMORY TO VESICULAR STOMATITIS VIRUS (VSV) IN MICE S.Charan* and R.M. Zinkernagel, Univ.Zurich, Zurich, Switzerland.

The concept of vaccination depends entirely on the existence of immunological memory and conventionally it is accepted to exist at both B and T cell level. We investigated T and B cell memory in mice immunized with VSV.

Two serotypes of VSV viz. Indiana and New-Jersey are distinct for neutralizing determinants whereas cytotoxic T lymphocytes induced are cross reactive. Mice were primed and challenged with homologous and heterologous serotype with cyclosporing-A (CS-A) treatment. (CS-A abrogates primary Ig response but the already primed cells are resistant to its effect). We observed that mice primed with homologous serotype (B cell) when challenged after 15 months with CS-A induced classical secondary response, whereas mice primed with heterologous serotype (T cell) showed an enhanced response only for a short time. We conclude that the objective of vaccination should be making B cells primed rather than T cells.

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R14.5

PRODUCTION OF INTERFERON BY LYMPHOCYTES AFTER VACCINATION WITH LIVE MEASLES VACCINE T. Nakayama, T. Urano, N. Maehara, K. Sasaki, and S. Makino. Department of Pediatrics, Saiseikai Central Hospital, and Department of Virology, The Kitasato Institute, Tokyo, Japan

We have reported that interferon (IFN)-γ was produced by T4 cells in the presence of macrophages in response to measles virus, and it was produced by both T4 and T8 cells without macrophages in response to mumps virus (J. Infect. Dis. May, 1987). Here, we have investigated the production of IFN by lymphocytes after vaccination with live measles vaccine and combined measles-mumps vaccine. IFN-α was produced before vaccination in response to the measles virus. Lymphocytes became hyporeactive in IFN-α production one week after vaccination, and this continued for 10 weeks. IFN-γ was produced by lymphocytes two weeks after vaccination and thereafter continuously in response to the measles virus. The levels of IFN-α stimulated by the mumps virus showed no significant change during the course of the vaccination. These results demonstrate that hyporeactivity in IFN-α production appears to be virus specific. As for vaccination with combined measles-mumps vaccine, antigen specific production of IFN-γ in response to both measles and mumps virus was observed two weeks after vaccination. Lymphocytes obtained from individuals without detectable HAI antibodies after vaccination produced IFN-γ, demonstrating the development of virus-specific cellular immunity.

R14.6

ANTIVIRAL ACTIVITY OF CHEMICALLY SYNTHESIZED LIPID A-SUBUNIT ANALOGUE (GLA-27). S. Ikeda, C. Nishimura[1], M. Nakatuka, J. Y. Homma[2], M. Kiso & A. Hasegawa[3]. School of Pharmaceutical Sciences, Kitasato University[1], Kitasato Institute[2], Tokyo, and Faculty of Agriculture, University of Gifu[3], Gifu, Japan.

Lipid A is known to be the active center responsible for most of the varied biologic[illegible] activities of lipopolysaccharide (LPS). Recently, we are interested in chemically synthesized analogues correspnding to the non-reducing sugar part of lipid A, if these compounds still remain the activity of lipid A. In the course of such investigations, we have found that most of the activities of Lipid A can be expressed in a structural analogue, GLA-27 (4-O-phosphoryl-D-glucosamine with 3-O-tetradecanoyl and 2-N-3-tetradecanoyloxytetradecanoyl groups). GLA-27 has very low toxicity and pathogenicity.

The investigations were carried out on the activities of GLA-27 to macrophage and NK cell activations and to IFN-inducing activity. These are related to non-specific resistance against viral infections. As a result, the activity of GLA-27 in vaccinia virus tail-lesion test was 100 fold higher than that of muramyldipeptide (MDP), a known immunomodulator. GLA-27 has an immunomodulator activity by enhancing non-specific host defence mechanisms.

R14.7

THE ROLE OF LOCAL T CELL SUBSETS IN RECOVERY FROM A CORNEAL HERPES VIRUS INFECTION. K.Hayashi, M.Kazami and Y.Uchida

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Department of Ophthalmology, Tokyo Women's Medical College, Tokyo, Japan 162

In order to study the relative importance of T cell subsets in controlling corneal herpes virus infections in mice, monoclonal antibodies(anti-Thy 1.2, Lyt1.2,Lyt2.2,L3T4) have been used to deplete specific T cell subpopulations which had been obtained from local lymphnodes at 7 days post subconjunctival virus inoculation. Adoptive transfer of the whole lymphnode cells to nu/nu mice provided almost complete suppression of the viral growth both in the cornea and lacrimal glands. The inhibitory effect exerted by these local lymphnode cells was approximately 10 times stronger than that of the spleen cells. Lyt2+ T cell subset was more effective in inhibiting viral growth than L3T4 positive cells. The site of primary HSV challenge influenced the phenotype of the cytotoxic cells. Whether or not they had been cultured in vitro with the stimulation of HSV antigen prior to adoptive transfer into the mice also had an effect.

R14.8

INTERACTION BETWEEN CYTOMEGALOVIRUS AND CLASS I HLA MOLECULES: ROLE IN VIRAL INFECTIVITY.

Jane E. Grundy, Jane A. McKeating and Paul D. Griffiths. Department of Virology, Royal Free Hospital School of Medicine, London NW3 2PF, England.

We have recently shown that cytomegalovirus (CMV) binds the host protein β_2-microglobulin (β_2m) from body fluids or cell culture media. Such binding conceals the viral antigenic determinants recognised by neutralising antibodies yet this virus is infectious. Our data suggests that CMV uses the bound β_2m to bind to cells. The addition of human β_2m, or a fraction of fetal calf serum corresponding to bovine β_2m, enhances the infectivity of CMV grown in cell culture under serum free conditions. Furthermore CMV and β_2m compete for the same binding sites on fibroblasts and other cell lines. In addition CMV was found to bind to a significantly greater degree to Raji cells expressing Class I HLA antigens than to Daudi cells lacking such expression. Enhancement of Class I antigen expression by treatment with γ interferon increased levels of virus binding.

Our results demonstrate that CMV can use Class I HLA molecules as a virus receptor. We propose that when coated with β_2m, CMV has the capacity to displace β_2m from the Class I HLA heavy chain-β_2m dimer on the cell surface and bind to cells. The fact that β_2m enhances infectivity suggests that such binding leads to productive infection of cells.

R14.9

IN VITRO ANALYSES OF LYMPHOCYTE RESPONSES TO ROTAVIRUS ANTIGEN

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Mitogenic response and specific immunoglobulin production to rotavirus antigen (Odelia strain, serotype 4) were obtained from human peripheral blood lymphocytes (PBL) and secondary lymphnode lymphocytes (SLL) in two adults and an infant. Rotavirus antigen induce blastogenic response of B cells but not of T cells. Antigen-specific immunoglobulin production was T cell-dependent. Recombinant IL2 enhanced antigen-specific immunoglobulin production. Specific IgG and IgM production were found in PBL and SLL, while, specific IgA production was observed only in SLL. Specific immunoglobulin production was more in lymphocytes in adults than in those in an infant.

Possibility of using rotavirus as an in vitro model of mucosal immunity (like influenza virus) is discussed.

R14.10

IMPAIRMENT OF POLYMORPHONUCLEAR AND MONONUCLEAR PHAGOCYTE FUNCTION IS CONCOMITTANT WITH ENHANCED BACTERIAL GROWTH AFTER TREATMENT WITH SUPERNATANT OF LYMPHOCYTES INFECTED BY ADENOVIRUSES AND HERPESVIRUS IN VITRO.

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Aspects of immunodepression due to latent DNA viruses were studied. Separated human T-lymphocytes/ly-s/ were infected by oncogenic and latent adenoviruses and HSV-1. After longer incubation cell- and virus-free supernatants were mixed to PMNL and MNPh cells swallowing living Staphylococcus aureus: Supernatant of ly-s infected by HSV-1 or Ad-H12 impaired phagocytic activity: MNPh lost its activity completely, but one third of PMNL phagocytosis remained after mixing with 100x concentrated supernatants. Decreasing effect of supernatants of ly-s infected by Ad-H5 and H8 was milder. The viruses themselves did not modify phagocytosis. Staphylococcal growth was enhanced significantly by supernatants of ly-s infected with Ad-H12 and H5. Division of Staphylococci continued within MNPh cells, but inside PMNL no division was observed. Characterization of mediators responsible for these effects shows polypeptide feature; no IFN-activity. The effects are dose-dependent.

R14.11

INDUCTION OF IMMUNE RESPONSE TO INFLUENZA VIRUS WITH ANTI-IDIOTYPIC ANTIBODIES. **E.M.Andere, G.Kapaklis, D.O.White. Department of Microbiology, University of Melbourne, Parkville, Victoria, Australia.**

Rabbit anti-idiotypic (anti-Id) sera were raised against 5 monoclonal antibodies (MAbs) to influenza virus A/Memphis/71 hemagglutinin (HA), subtype H3. The MAbs bind to different antigenic regions of the HA molecule as judged by their reactivity towards a panel of MAb-selected variants of Mem71 virus which bear single amino acid changes in HA. After removal by absorption of antibodies to constant regions of mouse immunoglobulins (Ig), the anti-Id sera were used to examine the idiotypic relationship between these 5 MAbs and a further 12 anti-HA MAbs. Each anti-Id serum blocked binding of the homologous Id to virus and was highly specific for the immunizing MAb, idiotypic cross-reactivity being seen only between MAbs with identical fine specificity for HA. When the Ig fraction of each anti-Id serum was used to immunize naive BALB/c mice, 2 of the 5 anti-Id preparations induced significant antibody responses to Mem71 influenza virus. These antibodies were HA-specific and, from their fine specificity towards mutant HAs with known single amino acid substitutions, were shown to be directed to the same antigenic site on HA as was the original MAb. Certain individual mice also produced a separate population of antibodies reactive with influenza viruses of a different subtype.

R14.12

IN VIVO STIMULATION OF MURINE NATURAL CELL-MEDIATED CYTOTOXICITY BY INFLUENZA HEMAGGLUTININ. M. Houde[a] and D.J.S. Wrona[a,b]. (a) Virology Research Center, Institut Armand-Frappier, Laval, Canada; (b) Biochemicals Division, Institut Armand-Frappier.

Recently, we reported that purified influenza neuraminidase can induce Natural Killer activity or cell-mediated cytotoxicity (CMC) in vivo and in vitro. The in vivo stimulation of CMC in BALB/c mice by influenza hemagglutinin (HA) was evaluated. HA, from recombinant virus A/USSR/92/77 (H1) x A/Prague/1/56 (N1), was purified by centrifugation on continuous sucrose gradient and affinity chromatography and then administered by intravenous route to BALB/c mice. After 24 hours, spleen cells were recovered, purified and tested for their CMC activity in a 4 hour ^{51}Cr-release assay using YAC-1 cells as targets. Doses of 2 to 10 ug of HA were tested. Five ug of HA produced the optimal stimulation (2,7-fold above the control CMC activity). This antigen-induced CMC was further increased (4,5-fold) by the use of a synthetic adjuvant, stearyl tyrosine (ST). ST alone had no effect on CMC activity. These, and our earlier results, suggest that both purified surface influenza glycoproteins have the potential to stimulate CMC in vivo and that this potential can be modulated by the use of an appropriate adjuvant.

R14.13

IN VIVO INDUCTION OF A CELLULAR GENE BY BOVINE LEUKEMIA VIRUS. G. Spies, R. Reeves, C. Buck. Departments of Microbiology, Biochemistry, Genetics and Cell Biology, Washington State University, Pullman, USA

A bovine mRNA has been identified whose copy number is increased in cattle persistently infected with bovine leukemia virus (BLV). BLV is a retrovirus characterized by the presence of a transactivating factor (pX) which positively regulates the viral LTR. The activation of cellular promoters by the BLV pX gene could account for the modulation of the mRNA we have observed. Sequencing of the cDNA reveals that the gene is not related to BLV or to known cytokines or lymphokines. The 3' untranslated tail does possess a copy of the $(TATT)_2$ repeat we have noted in the untranslated tail of interleukin-2 and other lymphokines. The modulation was detected by immobilization of the cloned cDNA on nitrocellulose. Labelled cDNA probes from fresh peripheral blood lymphocytes of BLV positive cattle and ConA stimulated lymphocytes of BLV negative cattle hybridize strongly to the cloned cDNA whereas probes from fresh BLV negative lymphocytes hybridized weakly. The cloned BLV induced cDNA is 2700bp long with a minimum 116 amino acid open reading frame, although sequencing has yet to define the precise boundaries of this potential protein coding region. We are currently investigating the modulation of this gene in the course of normal "T" lymphocyte activation and its presence in cells from BLV infected cattle.

R14.15

VIRUSES AS POLYCLONAL CYTOTOXIC T LYMPHOCYTE (CTL) INDUCERS. Hyekyung Yang and Raymond M. Welsh, Dept. of Pathol., Univ. of Massachusetts Med. Sch., Worcester, MA 01605 USA.

Alloreactive CTL's distinct from virus-specific CTL and activated natural killer (NK) cells were generated during acute infection of C57BL/6J mice with either lymphocytic choriomeningitis virus (LCMV) murine cytomegalovirus (MCMV), vaccinia virus (VV), or Tacaribe virus (PV). The alloreactive CTL_2 shared similar antigenic markers ($Thy\text{-}1.2^+$, $Lyt\text{-}2.2^+$, and asialo $GM1^-$) with the virus-specific CTL which appeared at the same time 7 days postinfection. These alloreactive CTL lysed allogeneic but not syngeneic or xenogeneic targets, and were antigenically and functionally distinct from activated NK cells. Infection of LCMV-immune C3H/St mice with VV, MCMV, or PV stimulated both challenge virus-specific and LCMV-specific CTL. In both systems, cold target competition studies indicated that the lysis was due to multiple subsets of CTL with distinct specificities and not due to challenge virus-specific CTL cross-reacting with different target cells. Hence, acute virus infections stimulate CTL's directed against other antigens. Size separation by centrifugal elutriation indicated alloreactive CTL and CTL against previous virus infections are contained in large blast-size cell fractions, suggesting that they have been stimulated to divide. This suggests that virus infections not only have the ability to polyclonally stimulate B cells, as previously shown, but also to polyclonally stimulate CTL.

R14.17

IMMUNE RESPONSE AND DISEASE INDUCED BY OCULAR INFECTIONS WITH MUTATED STRAINS OF HSV-1 (KOS). R. Hendricks, J Glorioso. University of Illinois, Chicago, and University of Michigan, Ann Arbor, U.S.A.

There is substantial evidence for the involvement of the immune system in the pathogenesis of HSV-1 corneal stromal lesions. Anterior chamber (AC) HSV-1 infection has been shown to induce cellular, but not humoral immune tolerance to HSV-1. We are using strains of HSV-1 with point or deletion mutations in the major surface glycoproteins (gB, gC, and gD) to determine which of these are involved in tolerance induction in this model. We are also determining the effect that tolerance has on the development of corneal stromal lesions. A/J mice are infected on the corneas with wild type (WT) HSV-1, followed within one hour by AC infection of the same eye with WT or mutant strains of HSV-1.

AC infection with WT HSV-1 abrogated the delayed hypersensitivity (DH), and cytotoxic responses induced by corneal infection alone, and inhibited the formation of stromal lesions. AC infection with a gC^- deletion mutant of HSV-1 abrogated the cytotoxic response, did not affect the DH response, but still inhibited the development of stromal lesions. Two HSV-1 KOS mutants with point mutations in gC (mar C 10.1, and mar C 11.1) resulting in resistance to monoclonal antibody neutralization, were used to further characterize the epitopes involved in the induction of DH suppression. Preliminary results suggest a dominant role for one epitope defined by the C11.1 mutant. Experiments designed to identify the epitopes involved in suppression of the cytotoxic response in this model are in progress.

R14.14

VIRUS SPECIFIC ANTIBODIES IN THE NASOPHARYNX AND TRACHEA OF PONIES AFTER PRIMARY AND SECONDARY INFECTIONS WITH INFLUENZA A/EQUINE/NEWMARKET/79 (H3N8). D.Hannant, D.W.Jessett, J.A.Mumford. Equine Virology Unit, Animal Health Trust, Nr Newmarket, Suffolk, England, CB8 7PN.

Exposure of ponies to a standardised dose of equine influenza by nebuliser proved a reliable and reproducible method of infection. Kinetics of virus specific antibody production was studied in naive and previously infected ponies using a radioisotopic antiglobulin assay. Nasopharyngeal and tracheal washes (taken at endoscopy) in sterile saline were concentrated and antibody levels measured with respect to the concentration of equine albumin in the sample to correct for variable dilution effects. Antibodies of the IgG_{ab}, IgM and IgA isotypes in the respiratory tract were compared with serum antibody levels. In naive ponies, local antibody was detectable by day 7 post infection and levels rose to a maximum at the end of the sampling period at day 14. Ponies which had been previously infected with influenza showed a typical secondary local antibody response when reinfected, in that high levels were detected by day 4. This was true for antibody in nasal and tracheal washes. A proportion of previously infected ponies were protected from clinical disease although serum antibody was low. The contribution of locally produced antibody to clinical protection in sensitised ponies is being investigated.

R14.16

DIFFERENTIAL MODULATION OF MHC CLASS I ANTIGEN EXPRESSION ON MOUSE BRAIN ENDOTHELIAL CELLS BY MHV-4. J. Joseph, R.L. Knobler, F.D. Lublin, M.N. Hart. Jefferson Medical College Philadelphia, PA, USA, University of Iowa College of Medicine Iowa City, IA, USA

Endothelial cells are a component structure of the blood brain barrier (BBB), and can interfere with virus entry into the brain. Virus infection of brain endothelial cells may be cleared by cytotoxic lymphocytes recognizing viral and specific MHC class I molecules on infected cells. Therefore, BALB/c mouse brain endothelial cells were infected with MHV-4 in culture, and both viral and class I (H-2D and H-2K) antigen expression was analyzed by flow cytometry for up to four days after infection. Compared to background expression endothelial cell viral and $H\text{-}2D^d$ antigens increased two-fold following MHV-4 infection, but, $H\text{-}2\ K^d$ antigen was selectively decreased, by 60%. These changes were not produced following contact with virus inactivated by exposure to UV-irradiation. In contrast, on MHV-4 infected macrophages, a thirty-fold increase in viral antigen expression over background was observed. Although also enhanced, differential expression of class I antigens was not found. These data suggest that limited viral antigen expression, and differential modulation of class I expression on brain endothelial cells may affect the host's ability to clear this virus, or to restrict its ability to infect the brain by breaking through the BBB.

R14.18

MONONUCLEAR CELLS FROM REINFECTED INFLUENZA SEROPOSITIVE MICE PRODUCE A FACTOR WHICH INDUCES HYPORESPONSIVENESS TO LYMPHOKINES. J. F. Sheridan and M. A. Beck. Dept. Medicine and Microbiol. and Immunol. The Ohio State University, Columbus, OHIO, USA.

Supernates from cultures of splenocytes obtained from influenza virus seropositive C57Bl/6 mice, reinfected with homologous virus (A/Bangkok/1/79), contained a dialyzable factor that inhibited IL-2 augmentation of natural killer (NK) activity and the expression of leukocyte migration inhibition factor (LIF). The dialyzable factor was produced early post reinfection, and was not detected in cultures from mice reinfected 6 days earlier, nor from seropositive mice that were not reinfected. Partial purification of the dialyzable factor over a Sephadex G-25 column revealed a molecular mass of approximately 3000 daltons. The partially purified factor was found to be sensitive to trypsin as well as to temperatures greater than 56°C and to pH 2 and pH 10. Addition of the partially-purified factor to cultures of CTLL-20 cells prevented interleukin 2 (IL-2) dependent proliferation in a dose dependent manner.

Exposure of polymorphonuclear cells, NK cells and CTLL-20 cells to the partially-purified factor induced a state of hyporesponsiveness to lymphokines. Re-exposure of seropositive mice to homologous viral antigen results in the induction of a factor which prevents the biological expression, but not production of lymphokines.

R14.19

GENETIC ASPECTS OF THE IMMUNOSUPPRESSIVE ACTIVITY OF MALIGNANT RABBIT FIBROMA VIRUS. David S. Strayer, Justin Mulloy, Katherine A. Laybourn, and Julian L. Leibowitz. Dept. of Pathology, Univ. of Texas, Houston, TX., 77030, U.S.A.

We studied the genetic basis of virus-related immunosuppression using malignant rabbit fibroma virus (MV). MV is a highly immunosuppressive lymphocytotropic leporipoxvirus. Genetically, MV is similar to Shope fibroma virus (SFV). SFV is neither immunosuppressive, nor lymphocytotropic. By transfecting using BamHI restriction fragments from MV, we developed recombinants between SFV and MV in which the immunosuppressive character of MV was transferred to SFV. Recombinant viruses were selected by replication in lymphocytes, a property of MV but not SFV. Of the several transfected MV BamHI restriction fragments, only the 10.8 kb "C" fragment induces SFV to behave like MV *in vitro*. Fragment C-containing recombinant viruses produce plaques like MV on RK-13 cell monolayers, rather than foci like SFV. These recombinants are highly immunosuppressive *in vitro* at multiplicites of infection (MOI) of 0.1. At lower MOI (0.01), the 71 recombinants isolated fell into 4 broad categories of immunosuppressive activity. Group I is highly immunosuppressive, like MV. Group IV is comparable to SFV (not suppressive) at this MOI. Groups II and III are intermediate. Southern blot analysis confirms that these recombinant viruses obtained have varying sized fragment C inserts. Further analyzing these recombinants, we will determine which gene(s) is sufficient for leporipoxvirus-induced immunosuppression.

R14.21

EARLY AND LATE INHIBITION OF LYMPHOCYTE PROLIFERATION BY MEASLES VIRUS. R. Salonen, J. Ilonen and A. Salmi. Department of Neurology, University of Turku, Turku, FINLAND, National Public Health Institute, Oulu, FINLAND and Department of Medical Microbiology and Infectious Diseases, University of Alberta, Edmonton, Alberta, CANADA

We studied the effect of two different strains of measles virus on lymphocyte blast transformation response. Infectious virus inhibited the proliferation response to PHA from the beginning of the culture when freshly isolated peripheral blood mononuclear cells were stimulated. However, measles virus stimulated the proliferation of a T-cell line in the beginning and the inhibition was a relatively late phenomenon associated with cell death. The early inhibition by the Edmonston strain of measles virus was shown to be monocyte-dependant by depletion studies with monoclonal antibodies. It was also related to interferon-alpha (IFN-alpha) production as the addition of anti-IFN-alpha to the cultures blocked part of the virus-induced inhibition and the addition of exogenous IFN was inhibitive to the response of uninfected cells. The late inhibition by the Edmonston strain was associated neither with monocytes nor with IFN-alpha but correlated to cell death in cultures. The early and late inhibition by the Halle strain of measles virus was extremely strong blocking the proliferation almost completely and was associated with cell death from the beginning.

R14.23

ENHANCEMENT OF A SECONDARY ANTIBODY RESPONSE TO VSV "G" GLYCOPROTEIN BY INTERFERON-γ TREATMENT AT PRIMARY IMMUNIZATION. ***Kevin P. Anderson,*** **Elizabeth H. Fennie, Tilahun Yilma. Genentech, Inc. South San Francisco, CA 94080 U.S.A., and Department of Veterinary Microbiology and Immunology, University of California, Davis, CA 95616 U.S.A.**

Administration of recombinant DNA-derived murine or bovine interferon-γ (IFN-γ) to mice or cattle in conjunction with immunization using purified envelope "G" glycoprotein of vesicular stomatitis virus (VSV) dramatically enhanced the magnitude of a secondary virus neutralizing antibody response following a subsequent exposure to the same antigen. Enhancement was statistically significant and occurred at relatively low doses of IFN-γ in the absence of any additional adjuvants. Enhancement of the primary neutralizing antibody response was not always observed, even in cases where enhancement of the secondary response was demonstrated. Cattle administered IFN-γ in conjunction with immunization were more resistant to a subsequent challenge with VSV than those immunized without IFN-γ treatment. IFN-γ treatment in conjunction with immunization protocols may therefore provide a relatively safe and effective way to enhance immune response and protection without the inflammatory side effects of currently available adjuvants.

R14.20

ANTIBODY RESPONSE TO HERPES SIMPLEX VIRUS GLYCOPROTEINS gB AND gD. D.I.Bernstein, L.M. Frenkel, Y.J.Bryson, and M.G. Myers. Gamble Inst. Med. Res., Cinti., OH, UCLA Sch. Med., L.A., CA, and Children's Hosp. Res. Fdn., Cinti., OH, USA.

The herpes simplex virus (HSV) glycoproteins gB and gD are important targets for both cellular and humoral immunity. We have evaluated the IgG and IgA antibody (ab) response to cloned preparations of gB and gD (R.L. Burke, Chiron Corp.) in patients using an ELISA assay. IgG ab to gD was not present in the acute sera from patients with true primary HSV infection but developed in all patients. Anti gD IgA ab developed in 2/6 patients with true primary HSV infection and was found in 3/4 patients with nonprimary HSV infections. IgG ab to gB was found in 5/6 acute specimens from patients with true primary HSV infection and in 3/9 specimens from another group of HSV seronegative patients. Since gB cross reacts with a Varicella Zoster Virus (VZV) protein, we also evaluated these specimens for VZV ab and found a correlation between anti-HSV gB and anti-VZV ab. IgG and IgA ab to gB were present in all specimens obtained 30 days or more after HSV infection. Peak IgA titers to gB (GMT = 26.3) were significantly higher than titers to gD (8.1) ($p < .01$) as were peak IgG titers (2754.2 vs. 323.6 respectively) ($p < .001$). The study of the relationship of the ab response to these glycoproteins and the natural history of the disease are now possible although cross reactions to other herpesvirus glycoproteins should be considered during analysis.

R14.22

HUMORAL AND CELLULAR RESPONSES TO HIV AND POLYPEPTIDES IN A MODEL SYSTEM. B. Wahren[1], E.M. Fenyö[2], F. Chiodi[2], R. Kurth[3], J. Ghrayeb[4], S. Putney[5], R. Gallo[6] and D. Bolognesi[7], [1]National Bacteriological Laboratory and [2]Karolinska Institute, S-105 21 Stockholm, Sweden; [3]Paul Ehrlich Institut, D-6000 Frankfurt, FRG; [4]Centocor, Malvern, PA. 19355; [5]Repligen, Cambridge, MA.; [6]National Cancer Institute, Bethesda, MD. 20892; [7]Duke University, Durham, NC. 27710.

A model system was established for studies of humoral and cellular immunity to HIV antigens in primary and reactivated infection and after vaccination. Macaques (Macaca fascicularis) were immunized with purified HIV, a cell extract rich in gp120 or polypeptides of cloned genes for parts of p24, gp41 and gp120 (pE3). Western blots best showed the appearance of antibodies to nucleocapsid protein while antibodies to higher molecular weight envelope glycoproteins were better demonstrated by radioimmunoprecipitation. With whole HIV, antibodies to p24 appeared first, and sometimes were the only ones to be demonstrable. Several immunizations with HIV were required to obtain antibodies to gp120, and the response was weak. (g)p41 also had a poor immunizing effect. IgG synthesis from B-cells in vitro was well demonstrable to whole HIV, and generally paralleled the antibody titers of sera after multiple immunizations. The HIV-specific lymphocyte proliferation response as measured by DNA synthesis was best seen with p24, followed by (g)p41, pE3, gp120 and whole HIV.

R14.24

THE IMMUNOMODULATING ACTIVITY OF CORYNEBACTERIUM PARVUM IN INFLUENZA INFECTED MICE. A.Denys, J.Białek, M.Kowalska, T.Szydłowska, Department of Microbiology, Military Medical Academy, Łódź, Poland

Our own investigation is based on the statement about the depression of cell mediated immunity during influenza infection. Phagocytic and bactericidal activity of granulocytes decreased, target cells infected by influenza virus are successfully destroyed by T lymphocytes. Corynebacterium parvum is a preparation up-to-now known as an anticancer drug, especially in metastasis of pleura and peritoneum. The aim of the study was to investigate the effect of the injection of killed suspension of Corynebacterium parvum in influenza virus infected mice.

C.parvum /Coparvax,Wellcome/ was injected intraperitoneally at a dose of 7 mg/kg body weight. We examined the parameters of humoral and cellular immunity 3,6,9,14 days after influenza infection: liberation of leucocytes inhibitory factor, the phagocytic and bactericidal activity of granulocytes. Injection of C.parvum gave as a result a stimulation of granulocytes activity during influenza infection. C.parvum normalized the disturbed function of the cells, taking part in the processes of immunological response of an organism.

R14.25

THE EFFECT OF VIRUS INFECTION ON I-A ANTIGEN EXPRESSION IN MURINE MACROPHAGES. L.-X. Wu, S.Vydelingum, R. Marusyk, A.Salmi. Department of Medical Microbiology and Infectious Diseases, Faculty of Medicine, University of Alberta, Edmonton, Alberta, Canada.

We have investigated the effects of Semliki forest virus (SFV) and measles virus (MV) infection on the expression of class II antigen (I-A) on murine peritoneal macrophages. A wild-type and a demyelinating strain of SFV induced I-A antigen expression in peritoneal macrophages within 24 hours after infection. The induction was dependent upon the infectious process as UV inactivated virus was non-inducing. However, both live and inactivated virus were able to decrease, in a dose-dependent manner, the percentage of I-A positive macrophages activated with ConA supernatants from rat spleen cells. The effect of the SFV infection on I-A expression was accompanied by decreased and delayed IL-1 production and abortive virus replication. All strains of MV examined were able to induce I-A antigen but with different kinetics of expression. Some MV strains decreased the percentage of I-A positive cells in an activated macrophage population while an inactivated ts mutant could synergistically enhance I-A antigen expression. Both the increasing and decreasing effects on the I-A expression may be through enhancing or suppressing mediator molecules not yet identified in this system. The results suggest that virus infection of monocyte-macrophage cells can have an up- or down-regulating role in the immune response.

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R14.26

IMMUNOSUPPRESSION IN BALB/C MICE BY INACTIVATED MEASLES VIRUS. Richard H. Smith, Mika J. Mäkelä, and Aimo A. Salmi., Viral Pathogenesis Research Unit, Department of Medical Microbiology and Infectious Diseases, University of Alberta, Edmonton, Alberta T6G 2H7.

After von Pirquet's observation in 1908 that an acute measles virus (MV) infection could disrupt the tuberculin-sensitivity reaction, numerous other reports have documented that a transitory immunosuppression caused by virus replication in lymphocytes occurs during a MV infection. We have found that purified ß-propiolactone inactivated MV can suppress the murine T cell immune response to inactivated herpes simplex virus (HSV) both *in vivo* and *in vitro*. If at the time of HSV immunization MV was added, the resulting HSV blastogenic response was suppressed up to 50 % as compared to the normal HSV proliferative response. However, if mice were cyclophosphamide (CP) pre-treated 2 days before HSV-MV immunization, the HSV response was not suppressed. If MV and HSV together were used to stimulate HSV immune lymphocytes *in vitro*, the resulting proliferative response was decreased 60 % when compared to HSV stimulation alone. This *in vitro* induced MV immunosuppression was not sensitive to CP pre-treatment. The *in vivo* and *in vitro* MV induced immunosuppression was virus-specific, and not due to cross-reactive cellular components, as neither purified inactivated vesicular stomatitis virus, mumps virus nor Vero cell lysate could suppress the HSV cellular immune responses. These results indicate that MV proteins have immunosuppressive properties. We are currently using purified proteins and glycoproteins to localize the suppressive protein(s) in MV.

This work was supported by the Alberta Heritage Foundation for Medical Research.

R14.27

PREDOMINANCE OF IgG2a IN MURINE HUMORAL IMMUNE RESPONSE ELICITED BY VIRAL INFECTIONS. J. van der Logt (1), J. Coutelier (2), F. Heessen (1), J. van Snick (3), Dept. of Med. Microbiology (1) University of Nijmegen, The Netherlands, Unit of Exp. Medicine (2), ICP and UCL, Ludwig Institute for Cancer Research (3), Brussel, Belgium.

The isotypic distribution of IgG antibodies was determined in the serum of mice after infection with a panel of RNA- and DNA viruses of eleven different genera. For comparison, mice were also immunized with a number of soluble protein antigens. The antiviral response induced by all these viruses showed a striking preponderance of the IgG2a subclass (65% to 92% of total antiviral IgG). A predominance of IgG1 was observed with soluble protein.

Concomitant viral infection (Lactodehydrogenase virus (LDV), mouse hepatitis virus, and mouse adenovirus) and immunization with soluble protein, or inactivated viral antigens resulted in an increased production of protein specific or antiviral IgG2a antibodies with a 26-fold increment of the IgG2a/IgG1 ratio for tetanus toxoid and a 150-fold increment for inactivated LDV.

These observations indicate that this IgG2a restriction is induced by the viral infection itself and strongly suggest the existence of highly specific mechanisms for the regulation of individual subclasses in the mouse.

R14.28

HUMAN CYTOMEGALOVIRUS SPECIFIC CYTOTOXIC T CELLS RECOGNISE NON-STRUCTURAL VIRAL PROTEINS. L.K. Borysiewicz, J. Hickling, Shek Graham, J.G.P. Sissons, MRC Clinical Immunology Research Group, RPMS. London, U.K.

Cytotoxic T (Tc) cells are important in maintaining the virus/host equilibrium in persistent virus infections. We have shown that human cytomegalovirus (HCMV) specific Tc, from normal persistently infected individuals, killed infected cells when non-structural antigens (IEA and EA) were expressed. The quantitation and stage specificity for the virus replicative cycle was determined by limiting dilution analysis of peripheral blood T cells from such subjects. T cells were grown in 30 replicate cultures at 10 cell concentrations (100-10,000), with IL2 and HCMV/VZV infected autologous fibroblasts. After 14d each culture was assayed to determine HCMV/VZV specific cytotoxicity. HCMV specific Tc were present at 1/5000-20,000 T cells, whereas VZV specific Tc were at 1/30,000-500,000. This may reflect the different sites of latency of α and β herpesviruses. Clonally derived Tc predominantly recognised IE/EA in cells inhibited following HCMV infection but <30% recognised structural antigens. The majority of Tc recognising non-structural antigens were specific for IEA. Thus HCMV specific Tc, like their murine counterparts, recognise IEA which maybe important in considering the future design of subunit HCMV vaccines.

R14.29

AN ANTI-IDIOTYPE MONOCLONAL ANTIBODY TO A PLANT VIRUS MONOCLONAL HAS FEATURES CHARACTERISTIC OF AN EPIBODY. R. L. Hernaugh, D. P. Durand, J. H. Hill*. Departments of Microbiology and Plant Pathology*, Iowa State University, Ames, Iowa.

S1 is a Balb/c derived, IgG2a κ monoclonal antibody (McAb) generated against soybean mosaic virus (SMV). A syngeneic, IgM κ, anti-idiotypic McAb was derived against glutaraldehyde-fixed, S1-secreting hybridomas. In ELISAs, 1a recognized intraspecies, cross-reactive idiotopes (CRIs) on S1 and 4F11 (an IgG2a κ McAb generated against Newcastle Disease Virus) and interspecies CRIs on protein A-purified, rabbit polyclonal antibodies (RAbs) to SMV. The 1a does not react with all IgG2a κ McAbs nor does it react with RAbs to two other plant viruses. Either S1 or SMV will compete with SMV RAbs for bound 1a in ELISAs indicating that S1 and SMV RAbs have nearly identical CRIs and that 1a recognizes an idiotope near the paratope of SMV RAbs. Competition between S1 and SMV for bound 1a could not be demonstrated. Furthermore, 1a recognized both S1 and SMV. The anti-idiotype 1a has not been affinity-purified against S1; however, due to the high level of reactivity 1a has with the idiotype S1 and the antigen SMV, 1a appears to exhibit features commonly associated with epibodies. These results indicate that epibodies may be involved in facets of the immune network outside of the autoantibody systems described thus far.

R14.30

ALTERATION IN IL-2 PRODUCTION AND EXPRESSION OF T CELL SURFACE GLYCOPROTEINS DURING VACCINIA VIRUS INFECTION OF CYCLOPHOSPHAMIDE-TREATED MICE. Z. Tabi, Jane E. Allan, R. Ceredig and P.C. Doherty. Dept. Experimental Pathology, John Curtin School of Medical Research, Australian National University, Canberra, Australia.

Recovery from poxvirus infection depends on the cytotoxic T cell response. However, the subclinical infection can be converted to lethal disease by treatment of mice with 300mg/kg of cyclophosphamide (Cy) prior to infection. This is accompanied by a loss of more than 80% of spleen cells and a decreased capacity, on a cell-for-cell basis, to develop virus-immune cytotoxic T lymphocytes (CTL) though the frequency of CTL precursors among surviving T cells is not greatly modified. Phenotypically, the surviving T cells express low levels of cell-surface Thy1, Lyt2 and L3T4 and, upon stimulation, are less able to produce IL-2 for more than a week following Cy treatment. The defect in capacity to generate CTL effectors both in vitro and in vivo can be corrected by providing an exogenous source of IL-2. These experiments indicate that a single dose of Cy induces changes in T cells that persist throughout the development of the immune response to viral infections.

R15.1

A NEW ANIMAL GENE-EXPRESSION VECTOR

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Sindbis virus is an enveloped animal virus, whose genome consists of a single-stranded RNA of plus polarity. As its naked genomic RNA is infectious and its structural proteins are very efficiently translated, Sindbis could be an excellent candidate for developing a new vector for expressing foreign genes in eucaryote cells.

The coding region for the structural proteins of a full length cDNA clone of Sindbis virus was replaced by a bacterial chloramphenicol acetyltransferase (CAT) gene. In vitro transcripts of this clone are efficiently self-replicated and produce active CAT when transfected into chicken and Drosophila cells. This genome can be packaged when cells are initially coinfected with helper Sindbis virus and it is stable for at least five cycles of replication.

This approach is not only useful for demonstrating the feasibility of a new efficient animal gene-expression vector, but also has proved valuable for studies on viral gene expression.

R15.2

HIGH-FREQUENCY DNA RECOMBINATION IN A VACCINIA VIRUS RECOMBINANT. L. Andrew Ball, Dept. of Microbiology, University of Alabama-Birmingham, Birmingham, AL 35294, USA.

In order to study homologous recombination of vaccinia virus DNA, we constructed a recombinant in which the viral thymidine kinase (tk) gene was flanked by direct repeats of a 1.5kb DNA sequence of heterologous origin. When forced to replicate in tk^- cells under tk^+ selective conditions, the recombinant maintained its tk^+ phenotype. Under tk^- or non-selective conditions, however, the tk gene was frequently excised because the repeated sequences provided substantial targets for homologous DNA recombination. Analysis of the DNA products showed that recombination readily occurred both inter- and intramolecularly. Unique DNA products of intramolecular recombination were detected in the cytoplasm of infected cells soon after the onset of viral DNA replication, and their appearance was blocked by inhibitors of DNA synthesis.

During repeated passage of the virus under non-selective conditions, the tk^+ fraction decreased with first-order kinetics at a rate that reflected the frequency of recombination per cycle of virus replication. Eventually, a residual population of stable tk^+ viruses remained, and analyses of the genome structures of individual members of this population showed that some of them appeared to be the products of non-homologous DNA recombination.

R15.3

WITHDRAWN

R15.4

INSECTS EXPRESSING THE HEMAGGLUTININ OF INFLUENZA VIRUS

K. Kuroda[1], A. Gröner[2], K. Frese[3], R. Rott[4], W. Doerfler[5] and H.-D. Klenk[1]
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The cloned hemagglutinin gene of fowl plague virus was introduced into the baculovirus AcNPV using homologous recombination techniques. Previously, biologically active hemagglutinin was detected in cultured insect cells infected with recombinant AcNPV (Kuroda et al., EMBO J. 5, 1359-1365, 1986). To express the hemagglutinin in whole insects, we have now infected larvae of tobacco budworm (Heliothis virescens). The hemagglutinin was synthesized in fat body, epidermis, and trachea, but not in the gut, indicating that the recombinant virus had the same tropism as authentic AcNPV. The hemagglutinin was correctly processed in these tissues into the fragments HA_1 and HA_2. Homogenates of infected larvae exhibited hemagglutinating activity and could be used for effective immunization of chickens against fowl plague.

R15.5

HIGH LEVELS OF EXPRESSION OF $p37^{mos}$ IN C-127 MOUSE CELLS. A. Seth[1], D. Blair[2], R.J. Fisher[2], K.J. Dunn[2], and G.F. Vande Woude[1]. [1]BRI-Basic Research Program, [2]Laboratory of Molecular Oncology; NCI-Frederick Cancer Research Facility, Frederick, Maryland, USA.

In order to study the biochemical activities associated with mos oncogene under physiological conditions, we have constructed a vector capable of expressing the protein product product at high levels in eukaryotic cells. In this vector the mos gene is expressed under the control of mouse metallothionein promoter and contains at its N terminus a 35 amino acid signal peptide derived from tissue plasminogen activator (t-PA). C-127 cells transformed by this mos construct express the mos protein at very high levels predominantly as a t-PA-mos fusion product ($p41^{mos}$), but no mos products are detected in the culture media. Lower levels of $p37^{mos}$ are produced in these cells presumably as a result of some processing of $p41^{mos}$, but the level of $p37^{mos}$ is still ~ 50-fold higher than observed in cells transformed by v-mos or c-mos. Cellular localization studies show that the product is present in the membrane fraction as well as in the cytoplasm and we suspect this unusual distribution is why very high levels are tolerated. We have purified soluble $p41^{mos}$ and $p37^{mos}$ and are characterizing its biochemical properties.

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R15.6

PRODUCTION OF RECOMBINANT COLONY-STIMULATING FACTOR-1 (rCSF-1) BY SV40 VIRUS INDUCTION IN CV-1 CELLS IN SERUM-FREE MEDIUM. S. Weiss and J. Weaver. Cetus Corporation, Emeryville, CA, USA.

An optimized production system utilizing multiple harvests has been developed for rCSF-1 expression. A CSF-1 cDNA-containing plasmid (Kawasaki et al., Science, Vol. 230, 291-296, 1985) under the control of SV40 virus regulatory elements was stably transfected into CV-1 monkey kidney cells. Confluent cultures of those CV-1pCSF-17 Neo cells when infected with SV40 virus at a multiplicity of infection of 0.5 expressed 2.75×10^6 units of rCSF-1 per 200 ml of culture using the multiple harvest method. Harvested and pooled serum-free and protein-free supernatant containing rCSF-1 was clarified by centrifugation-filtration prior to purification, with no significant loss of rCSF-1 based on radioimmunoassay. Clarified rCSF-1 has been purified to homogeneity and shown to be effective in a murine tumor model and an infectious disease models in vivo, as well as being active in a mouse bone marrow proliferation assay producing colonies of predominantly macrophage morphology.

R15.7

INSERTION OF TOBACCO RATTLE VIRUS RNA-2 C-DNA INTO A TRANSCRIPTION VECTOR. D. Pena, A. Siegal. Department of Biological Sciences, Wayne State University, Detroit, MI U.S.A.

Tobacco Rattle Virus (TRV) is a bipartite virus composed of a long and short rod each containing one plus stranded RNA molecule. The nucleotide sequence of the smaller genomic strand (RNA-2) has been previously determined. We report the insertion of a full length DNA copy of RNA-2 into the transcription vector pBLUEM13 (bluescribe). This was accomplished by synthesizing the cDNA to RNA-2 using synthetic oligonucleotide primers with 5' extensions. These extensions were able to annealto bluescribe termini that had been generated by digestion with appropriate restriction enzymes. Completeness and accuracy of the insertion was assessed by determining its terminal nucleotide sequences. The insert containing plasmid (pDP1) was further modified by deleting a portion of the multicloning region so as to bring the insert to 7 nucleotides from the T7 promoter transcriptional start site. Runoff transcription of the resulting plasmid (pDP2), cleaved precisely at the 3' prime end of the insert, yields RNA of the same size as RNA-2 as judged by denaturing agarose gel electrophoresis. The nature and biological activity of this synthetic RNA are under current study.

R15.8

USE OF E.COLI PLASMID FOR THE DETECTION OF PROMOTERS FROM VACCINIA VIRUS TIAN TAN STRAIN GENOME. Hou Yunde, Yu Wenhao and Gin Qi
Institute of Virology, Chinese Academy of Preventive Medicine.Beijing, China.
Six vaccinia virus early and/or late promoters and more than twenty DNA fragments from vaccinia virus Tian Tan strain genome(Chinese vaccine strain)were shown to be able to initiate transcription of chloramphenicol acetyltransferase (CAT) gene in E.coli to make them the chloramphenicol resistant phenotype. This finding stronly suggests that the procaryotic RNA polymerase is able to recognize the vaccinia virus promoter. The utilization of E.coli plasmid to isolation of vaccinia virus promoter appears a simple, direct and effective method and may be useful in the study on the regulation mechanism of vaccinia virus gene expression and construction of vaccinia virus vector.

R15.9

USE OF A RETROVIRAL VECTOR TO ANALYZE ACTIVATED FORMS OF THE HUMAN C-SRC GENE PRODUCT. Akio Tanaka++, John Bechberger+ and Donald J. Fujita++. Cancer Research Laboratory, Univ. of Western Ontario, London, Ont.+, and Dept. of Medical Biochemistry, Univ. of Calgary, Calgary, Alberta+

In order to facilitate studies on the human c-src proto-oncogene, we have utilized a replication-competent retroviral vector (derived from Rous sarcoma virus DNA) with two types of inserts: A) the genomic form of human c-src (19kb) (WO CS) , and B) a chimeric src gene formed between the human c-src gene and the v-src specific sequence encoding the COOH-terminal v-src-specific 12 amino acids (WO CVS). The WO CS virus, containing the normal human c-src gene, does not cause cell transformation, whereas the WO CVS virus induces cell transformation. In addition, 4 spontaneously arising transforming variants that induce distinct transformed cell morphologies were isolated from cultures of CS-infected cells. Molecular cloning and DNA sequencing of proviral DNAs indicated the following: 1) The 10 introns of the genomic c-src gene were successfully spliced out; 2) The DNA sequence of the c-src gene in the CS virus was identical to that of the genomic c-src coding sequence; this is the the first example of a successful isolation of an intact c-src cDNA; 3) CVS and one mutant src gene exhibited similar lesions abolishing a COOH-terminal regulatory domain, which parallels an important step in the generation of the RSV v-src gene; 4) Other mutant src genes exhibited point or deletion mutations that would result in drastic conformational changes in the c-src protein. Possible activation mechanisms for src proteins will be discussed.

R15.10

DECREASED EXPRESSION OF THE T4 ANTIGEN ON CD4+ CELLS INFECTED WITH RECOMBINANT VACCINIA VIRUS ENCODING THE HIV ENVELOPE GENE. Y. Rivière, M.P. Kieny, P. Salmon, J.C. Gluckman, D. Klatzmann and L. Montagnier, Institut Pasteur, Paris ; Transgène, Strasbourg ; UER Pitié-Salpétrière, Paris, FRANCE.

The infectivity of HIV, the aetiological agent of acquired immune deficiency syndrome, seems to be generally restricted to cells expressing the T4 glycoprotein on their surface and this property has been linked to a specific interaction of the HIV envelope 110Kd glycoproteins with the T4 molecules on the cell surface. Furthermore, CD4+ cells infected with HIV show decreased expression of the T4 antigen on their surface.

Expression of the HIV envelope gene by a recombinant vaccinia virus in infected CD4+ cells provides us a tool to study the gp110-T4 interaction. CD4+ cells were infected in vitro with vaccinia virus recombinant encoding the HIV/LAVI env gene and expression of various membranes markers together with the gp110 envelope antigen was followed using a fluorescence activated cell analyser. Progressive and complete disappearance of Leu 3a staining associated with a 3 to 10 fold decrease of OKT4 fluorescence intensity correlated with increasing expression of the gp110 antigen. Thus, there is a direct correlation between the reduction of CD4 expression and the increase of gp110 expression on the surface of CD4+ cells infected with a vaccinia vector encoding the HIV env gene in the absence of other HIV genes expression.

R15.11

EXPRESSION OF BLUETONGUE VIRUS STRUCTURAL PROTEINS IN A BACULOVIRUS EXPRESSION VECTOR. Polly Roy, S. Inumaru* & J.J. Marshall*. Department of Environmental Health, University of Alabama, Birmingham, Al 35249, U.S.A. and *Institute of Virology, Mansfield Road, Oxford, OX1 3SR U.K.

Bluetongue virus, a member of the Reoviridae family, contains a genome of 10 double-stranded RNA segments each of whichis unique and believed to code for a single polypeptide product. The genome is surrounded by an inner core of five polypeptides (VP1, VP3, VP4, VP6 and VP7), which in turn is surrounded by an outer capsid of two polypeptides (VP2 and VP5).

In order to define the function of each protein, DNA representing RNA segments of BTV-10 have been inserted individually into a baculovirus transfer vector in lieu of the 5' region of the polyhedrin gene of Autographa californica nuclear polyhedrosis virus (AcNPV). After cotransfection of insect cells with wild-type AcNPV DNA in the presence of derived recombinant transfer vector DNA, recombinant virus was recovered. In this presentation, the expression of these polypeptides will be discussed, each of which was demonstrated to be synthesized in insect cells in high levels. Antibodies raised in mice or rabbits to the baculovirus expressed proteins immunoprecipitated the respective BTV antigens.

R16.1

PREDICTION AND COMPARISON OF SECONDARY STRUCTURES AND SURFACE HYDROPHILIC PROBABILITIES FOR POLIOVIRUS CAPSID PROTEINS. Wang Hong, He Jun, Yang Xiaofeng, Guo Ren. Institute of Medical Biology, Chinese Academy of Medical Sciences, Kunming, CHINA.

The all capsid proteins (Vp1, Vp2, Vp3, Vp4) amino acid sequences of poliovirus 1,2,3 types 6 strains were analyzed with Chou-Fasman method and Janin method on computer to predict and compare its secondary structure and surface hydrophilic probabilities.

The results showed; (1). β turns were more than helices and β sheets in all capsid proteins of poliovirus, specialy in Vp1. (2). The β turn was considered as keeping space structure of capsid protein and as structure basis of epitope. 1 or 2 β turns Could form an epitope. (3). Secondary structure was no change intratypies. Attenuation could basically be caused by the change of third structure. (4). Surface hydrophilic probability of Vp1, Vp2, Vp3 was different intertype, specially in 2 type. Main epitope (the highest peak) of all capsid protein was sited in different region. (5). The spacific peaks were not same. The common peaks were very similar intratype and nearing intertype. It provided thory basis for seeking the intertype and intratype common epitope of poliovirus.

R16.2

STUDIES ON MOLECULAR EVOLUTION OF THE PICORNAVIRIDAE. Yang Xiaofeng, Wang Hong, Li Ming, Zhai Chaoyang, He Jun, Guo Ren. Institute of Medical Biology, Chinese Academy of Medical Sciences, Kunming, China.

First attempt to compare and align the amino acid sequences of 14 virus RNA polymerases of four genera in the picornaviridae by means of Lipman's algorithm of searching protein homology in computer was made. The results showed: (1) RNA polymerases of 5 polioviruses were highly conservative, with above 97% homology, those of 4 FMDV also with above 95% homology; (2) those between Cox B3 virus and polioviruses were above 73%; (3) comparison between 2 rhinoviruses and others and between encephalomyocarditis virus and others suggested that previous classification about these viruses be correct; (4) homology between hepatitis A virus and other 13 viruses were only in a range of 13.83-19.52%. This results suggested that it be not exact for HAV to be classified as type 72 of enteroviruses and HAV should be classified as a single genus of the picornaviridae. An evolutionary tree of picornaviruses representing the relationship between 14 viruses was first drawn. The comparison of space structure of RNA polymerases by means of Janin's method calculating protein surface probability supported the results of homology comparison of amino acids of 14 RNA polymerases.

R16.3

SITE-SPECIFIC MUTAGENESIS OF A PICORNAVIRAL 3C CLEAVAGE SITE. G.D. Parks and A.C. Palmenberg. Biophysics Laboratory, University of Wisconsin, Madison, WI, U.S.A.

In order to express multiple proteins from a single genomic RNA, picornaviruses rely on cleavage of polypeptide precursors by the viral 3C protease. Normally, the 3C enzyme from Encephalomyocarditis virus (EMC) processes its polyprotein at Gln/Gly or Gln/Ser amino acid pairs.

We have constructed cDNA plasmids which efficiently express the EMC capsid region precursor polypeptide in vitro. Specific mutations were introduced into the DNA encoding the Gln/Gly pair at the VP3/VP1 cleavage site. Protein precursors produced from these clones were assayed for their ability to be processed by 3C enzyme. We show that mutations resulting in Gln/Ala or Glu/Ala were recognized and cleaved by 3C. Protein precursors containing alterations to Lys/Ala, Lys/Glu or Pro/Gly were not processed. We also show that mutants defective in the VP3/VP1 site were not able to form 14S pentameric structures, the first step in assembly of picornaviral capsids.

R16.4

MOLECULAR ANALYSIS OF REPLICATION-DEFECTIVE MUTANTS OF POLIOVIRUS. K.M.Kean, H.Agut, O.Fichot, and M.Girard Molecular Virology, Pasteur Institute, Paris, France.

Complementary DNAs of poliovirus temperature-sensitive plurimutants were synthetized and mutated sequences introduced into the full-length infectious wild-type cDNA. Mutations were precisely located and identified by sequencing of genomic RNAs and cDNAs.

In the viral protease (3C) gene, a U to C change at n5658 substituted a threonine for an isoleucine. Analysis of protein synthesis in infected HeLa cells and of 3C expression in a bacterial system showed that this resulted in defective autocatalytic cleavage of 3C from its precursor at the C-terminus.

In the viral replicase (3D) gene, an A to G change at n7256 changed an asparagine to an aspartic acid. No detectable replication occurred in cells infected at 39°C but replicase synthetized at permissive temperature was active at 39°C in vitro. The mutation had a profound effect on computer - predicted RNA secondary structure. A C to U change at n7348 had no effect on the amino acid sequence of polypeptide 3D and, alone, had no phenotypic effect on the virus. However when this mutation was associated with the protease mutation onto a single genome, the effects of the protease mutation in vivo were partially suppressed .

R16.5

MUTATIONS IN THE 5' NON-CODING REGION OF POLIOVIRUS RNA. S. L. Dildine, S. L. Semler, Department of Microbiology and Molecular Genetics, University of California, Irvine, USA.

The poliovirus genome encodes a 5' untranslated region of ~750 nucleotides. This region constitutes 10% of the entire genome and as yet has no ascribed function. In order to determine the function(s) of the 5' non-coding region, we have introduced a number of short (2-4 bp) deletions and insertions at various locations in this region. Such mutations in the poliovirus genome are achieved via manipulation of sub-genomic cDNA clones which are subsequently reconstructed into full-length cDNA clones. Full-length poliovirus cDNA has been shown to produce infectious virus upon transfection of primate cells.

Full-length poliovirus cDNA clones containing the following mutations were constructed: 1) 4 bp deletion and insertion at the BamHI site at nucleotide 220, 2) 4 bp deletion and insertion at the BamHI site at nucleotide 670, 3) 2 bp deletion at the BamI site at nucleotide 456, 4) 4 bp insertion at the KpnI site at nucleotide 66. Transfection of the mutated cDNA clones into COS cells has produced infectious virus from several different plasmids. The insertion and deletion of 4 bp at the BamHI 220 site has produced temperature-sensitive (ts) viruses, designated Sel-5NC-F1 and Sel-5NC-D1, respectively. One of these viruses (Sel-5NC-F1) appears to revert to a wild type phenotype upon further passage. Both the insertion and deletion mutations at the BamHI 670 site have yielded infectious viruses, Sel-5NC-F2 and Sel-5NC-D2, respectively. Preliminary studies suggest that these viruses are not ts for growth at 37°C.

R16.6

NATURAL POLIOVIRUS RECOMBINANTS ISOLATED DURING AN EPIDEMIC IN CENTRAL AMERICA, 1984-1985. R. Rico-Hesse, M. Figueroa, O. Kew. Division of Viral Diseases, Centers for Disease Control, Atlanta, Georgia, 30333, U.S.A., and National University of Honduras, Tegucigalpa, Honduras.

Primer extension sequencing has allowed us to monitor the worldwide distribution of wild type 1 poliovirus genotypes. During these studies, a series of type 1 isolates from poliomyelitis patients, obtained during the 1984-85 epidemic in Honduras, were found to have recombinant genomes. Genomic sequences encoding the capsid region were nearly identical (<2% divergent) to those of the Sabin 1 vaccine strain used in national immunization campaigns. The noncoding and noncapsid sequences were highly divergent (up to 20%) from Sabin 1, and appeared to be derived from a wild type 1 poliovirus. Recombinant polioviruses were isolated early in the epidemic, but isolates from cases occurring late in the epidemic were nonrecombinant wild type 1 polioviruses. The distribution of the two genotypes within the population in Honduras were determined by use of specific DNA hybridization probes. To our knowledge, this is the first report of (1) the epidemic cocirculation of two distict poliovirus genotypes of the same serotype, (2) the participation of wild polioviruses in recombinational events in nature, and (3) the association of natural recombinants with epidemic poliomyelitis.

R16.7

MOLECULAR IMMUNOLOGY OF POLIOVIRUS BASED ON ISOLATED STRUCTURAL POLYPEPTIDES AND THEIR ANTIBODIES. R.Dernick, J.Heukeshoven, J.Hinze, H.Uhlig, K.J. Wiegers. Heinrich-Pette-Institut an der Univers. Hamburg, Martinistr.52, D-2000 Hamburg 20, F.R.G.

The structural analysis of picornaviruses by Rossmann et al. (Nature 317, 145, 1985) and Hogle et al.(Science 229, 1358, 1985) made these viruses the best subjects for studies on the structure and antigenicity of naked animal viruses. The rigidity and complex structure of these virions makes the elucidation of their antigenicity rather difficult.

We have developed effective methods for the separation of the structural proteins (VP) of poliovirus VP1,2,3 and 4 for the three serotypes. We have used VPs and virions for the production of monospecific rabbit antibodies and of murine and human monoclonal antibodies including a sophisticated in vitro immunization system and EBV transformed human B cells. The specific antibodies enabled us to undertake systematic studies of the antigenic determinants of poliovirus particles and their precursors and of the immunogenicity of isolated, viral polypeptides VP1, VP2 and VP3. Differences were found in neutralizing and binding antibodies depending on the immunized host, virus strain and polypeptide.

R16.8

THE FORMATION OF 100S PARTILES DURING POLIOVIRUS UNCOATING

L. Everaert, R. Vrijsen and A. Boeyé
Vrije Universiteit Brussel, Dept. of Microbiology and Hygiene. Laarbeeklaan 103, B- 1090 Brussels (Belgium)

The formation of RNA containing 135S particles and of 80S empty capsids as a result of poliovirus uncoating in HeLa cells is well documented. When uncoating was synchronised by temperature shift-up, we observed the formation of an additional species of particles sedimenting at approximately 100S. Like 135S, the 100S partles contain RNA and can be freed of cellular debris using detergents.
Temporal relations suggest a precursor-product relation between 135S and 100S particles and between 135S and 80S empty capsids but not between 100S and 80S (although this possibility can not be excluded).
The possible *in vitro* uncoating of 135S and 100S particles will be studied to find out wether theseare biologically relevant, or dead-end products

R16.9

THE EFFECT OF A PROTEOLYTIC CLEAVAGE SITE ON THE ANTIGENIC VARIATION OF POLIOVIRUS STRAINS. M Ferguson, D I Magrath, P D Minor. Division of Virology, National Institute for Biological Standards and Control, London.

Antigenic variation among poliovirus strains has been studied by examining the reactivity in neutralisation assays of monoclonal antibodies directed against each of the major antigenic sites with wild strains isolated in various parts of the world over the past thirty years. Differences between serotypes have been observed in the extent of conservation of individual antigenic sites suggesting that different sites on each serotype are recognised by the human immune response. The role of a trypsin sensitive cleavage site which is present in VP1 at amino acids 98 or 100 on some poliovirus strains has also been investigated as this site is known to be cleaved in virus present in faecal specimens from primary vaccinees.

R16.10

MOLECULAR IMMUNOLOGY OF POLIOVIRUS BASED ON ISOLATED STRUCTURAL POLYPEPTIDES AND THEIR ANTIBODIES. R.Dernick, J.Heukeshoven, J.Hinze, H.Uhlig, K.J. Wiegers. Heinrich-Pette-Institut an der Univers. Hamburg, Martinistr.52, D-2000 Hamburg 20, F.R.G.

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R16.11

MOLECULAR DIVERSITY OF HUMAN RHINOVIRUSES. G. Stanway, P.J. Hughes, C. North and P.D. Minor*. Department of Biology, University of Essex, Colchester and *NIBSC, London, UK.

Human rhinoviruses are the major causative agents of the common cold and one of the important determinants of the frequency of this disease is the large number of rhinovirus serotypes. The molecular basis of serotype diversity and the relationship between different rhinoviruses is, however, poorly understood. To investigate these areas we have undertaken a programme of comparative nucleotide sequencing. To date we have determined the complete sequences of serotypes 14 and 1B and partial sequences of serotypes 45, 72 and 85. Comparisons between them indicate that there is a great deal of diversity within the genus but also distinct genetic lineages exist. For example, serotypes 14 and 72 are closely related (>85% amino acid homology) but are much less homologous to serotypes 1B and 85 (50%). Sequences in the capsid region correlate well with receptor grouping and point to areas which should be studied further to assess their role in receptor binding.

R16.12

THE NUCLEOTIDE SEQUENCE OF COXSACKIEVIRUS A21. P.J. Hughes, C. North, P.D. Minor* and G. Stanway. Department of Biology, University of Essex, Colchester, and *NIBSC, London, U.K.

The coxsackie A viruses cause a wide variety of human diseases. As part of an investigation into the molecular basis of this pathogenicity we have determined the complete nucleotide sequence of coxsackievirus A21 (CA-21), a virus which causes an upper respiratory tract infection similar to that produced by the rhinoviruses and which shares a cellular receptor with this group of viruses.

The nucleotide and predicted amino acid sequences of CA-21 are remarkably similar to those of the polioviruses especially in the 3' part of the genome (the polymerase gene is 97% homologous). The capsid proteins are rather more diverse (VP1 is only 53% homologous) presumably accounting for the distinct pathogenicity and receptor binding properties. There is much less homology to sequenced rhinoviruses even in the areas postulated to be involved in receptor binding. The results point to a relatively recent divergence from the polioviruses but shed little light on the receptor binding properties of the virus.

R16.13

VIRAL PROTEIN PROFILES OF ECHOVIRUS 6 RECOVERED FROM LYTIC AND NON-LYTIC STEADY STATE INFECTIONS. R. V. Blackburn and V. F. Righthand. Dept. Immunol. Microbiol., Wayne State U., Detroit, U.S.A.

We established a persistent infection of cultured human cells by the normally cytolytic echovirus 6. These cultures contain genome size viral RNA and continuously produce virus particles without undergoing lysis. The virus particles recovered from persistently infected cells differ in their capsid proteins from virus particles recovered from lytic infections. The viral structural polypeptides were visualized by silver stain and fluorography after SDS-PAGE. These polypeptides were demonstrated to be viral by their immunoprecipitation with anti-echovirus 6 serum. The polypeptides isolated from lytic infections had apparent molecular weights of 31.5, 27, 25.8, 21 and 9.5 kd and were designated as VP0, VP1, VP3, VP2 and VP4, respectively. VP0 was often absent from fully infectious particles and its presence coincided with lower concentrations of the two smaller polypeptides suggesting a precursor relationship of VP0 to VP2 and VP4. Peptide analysis by partial proteolysis of isolated viral proteins provided further support for this suggestion. Only three polypeptides (VP0,VP1,VP3) were detected in particles isolated from persistent infections. These particles contained genome size viral RNA, but were non-infectious because of their inability to attach to uninfected cells.

R16.14

ANTIGENIC SITES ON FOOT AND MOUTH DISEASE VIRUS. D. McCahon, Q. Xie, J. Crowther, G. Belsham, Animal Virus Research Institute, Pirbright, Surrey, GU24 ONF, U.K.

Seven neutralising monoclonal antibodies were used to characterise 30 escape mutants of a type O foot and mouth disease virus (O_1 Kaufbeuren). Three nonoverlapping antigenic sites were found by ELISA and cross-neutralisation studies. All three sites were conformation dependent but one was less conformation dependent since the appropriate monoclonal antibodies were able to bind to 12S subunits, isolated VP1 and a synthetic peptide containing residues 141 to 160 of VP1 in ELISA. Primer extension sequencing of most or all of the VP1 coding region of 10 escape mutants of that site plus three parental isolates showed that at least five amino acids were involved. In only one case (residue 148 of VP1) did change at that residue produce complete resistance to neutralisation. Partial resistance was produced by change at residues 144, 154 or 208 of VP1 or another residue(s), as yet undefined, that is probably in one of the other capsid polypeptides. Thus the site defined by these mutants is made up of at least three regions, the region involving residues 144 to 154 of VP1, the region encompassing residue 208 from the COOH terminus of VP1 plus a region, probably of VP2 or VP3, encompassing the undefined residue(s).

R16.15

THE SYNTHESIS OF A PARTICLE-FORMING CELLULAR PROTEIN IS ENHANCED BY MENGO VIRUS INFECTION. Ulrike Boege, Ramona Hancharyk and Doug Scraba. Dept. of Biochem., Univ. of Alberta, Edmonton, T6G 2H7 Canada.

We have detected a cellular protein which not only escapes the shutoff of host translation induced by Mengo virus, but is synthesized in increasing amounts during Mengo virus infection. The protein has an apparent MW of 20,000 and is contained in a remarkably stable cytoplasmic particle with a sedimentation coefficient of ~16S in sucrose gradients. In the electron microscope this particle appears as a sphere of ~12 nm diameter. The synthesis of the protein is stimulated in mouse L-cells and in HeLa cells infected with Mengo virus. Its synthesis cannot be induced, however, by stress (heat) or by infection with reovirus.

Given that reovirus also effectively inhibits host mRNA translation and produces its own non-capped mRNAs during infection, it is unlikely that the 20 KD protein is produced from a cellular mRNA which is so similar in 5' secondary and tertiary structure to Mengo RNA that it is fortuitously translated after shutoff. It is more likely that this protein plays some role in Mengo virus replication.

R16.16

COMPARATIVE STUDIES OF THE DIABETOGENICITY OF THE D-VARIANT OF ENCEPHALOMYOCARDITIS (EMD-D) VIRUS IN MICE WHEN THE VIRUS IS INTRODUCED BY ARTIFICIAL OR NATURAL ROUTES OF INFECTION. J.H. Huffman, R.W. Sidwell, and R.P. Warren. Dept. of Animal, Dairy and Veterinary Sciences and Dept. of Biology, Utah State University, Logan, Utah, USA.

EMC-D virus was given to SJL/J inbred mice by the artificial, intraperitoneal (i.p.), route of infection or by the natural routes of infection, per os (p.o.) or intranasal (i.n.), in comparable concentrations. Various parameters of infection and of diabetes induction were observed in each. None of the mice given virus by the p.o. route developed signs of diabetes nor gave evidence of having experienced a generalized infection by the virus. In contrast to these results, mice given similar amounts of virus by either the i.p. or i.n. routes of infection yielded high percentages of conversion to diabetic signs as well as high titers of neutralizing antibody in their plasma. Mice inoculated by the i.n. route usually lagged one day behind those given virus by the i.p. route in glucose spillage in the urine. They were also better able to eliminate the virus prior to development of either diabetic signs or circulating antibody sufficient to neutralize EMC-D virus in vitro. Higher titers of virus were required by the i.n. than by the i.p. route to induce 100% of the mice to diabetic signs. The i.n. route was used to study effects of purification of the virus and to determine some resistance and infection parameters evident post virus inoculation.

R16.17

SEROTYPES OF ENTEROVIRUSES ISOLATED FROM NORMAL PIGS IN POLAND. J.Buczek, T.Kozioł. Department of Microbiology, Faculty of Veterinary Medicine, Agricultural University of Lublin, Po - land. Veterinary Diagnostic Center in Wrocław, Poland.

The occurrence and antigenic differentiation of viruses from the alimentary tract of pigs was studied using primary pig kidney cell cultures, and continuous cell lines of PK 15 and IBRS:. Forty five viral strains were isolated from feaces of pig from various regions of Poland. The isolates were classified on the basis of physicochemical examinations to Picornaviridae, genus Picornavirus. Further identification and serologic differentiation of the isolates was done by SN test using : 11 strictly determined serotypes of pigs enteroviruses from international collection and respective antisera /Dr.Knowles, The Animal Virus Institute Pirbright/ and the antisera against the reference strains and against the own isolates prepared in this Department.

It was found for the first time in pigs in Poland are present serotypes 2,3,6,7,8 and 9 of pig enteroviruses. Their role is under study.

R16.18

EXPRESSION OF HEPATITIS A VIRUS MAJOR SURFACE ANTIGEN IN E. COLI. J. Fleming, I. Kelberman, J. Hughes, D. Linemeyer, and S.W. Mitra. Merck Institute for Therapeutic Research, P.O. Box 2000, Rahway, NJ 07065.

The hepatitis A virus (HAV) is a member of the genus enteroviridae within the picornavirus family. The viral structural proteins; VP1, 2, and 3 have been identified. The HAV genome has been cloned by our laboratory and by others. The nucleic acid sequence surrounding the viral structural proteins has been determined and it was found to contain a single open reading frame against which a partial amino acid sequence was aligned. Cross-linking studies using monoclonal antibodies have demonstrated that VP1 contains at least one of the major neutralization sites. We have separated the genes encoding VP1 from the replicative and infective machinery of the viral genome and have expressed it in E. coli. Using antisera raised against both native and denatured HAV, we have shown that a VP-1 fragment and a VP1-VP3 fusion protein are both expressed. Of particular interest is the observation that post-covalescent marmoset serum is capable of reacting with VP1-VP3 fusion protein.

R16.19
NEUTRALIZING ANTIGENIC SITES ON TYPE A FOOT-AND-MOUTH DISEASE VIRUS DEFINED BY NEUTRALIZATION-RESISTANT VARIANTS.
B. Baxt, D.O. Morgan, V. Vakharia, and D.M. Moore. USDA-Plum Island Animal Disease Center, Greenport, NY, USA.

We have previously described seven neutralizing monoclonal antibodies (nMAb) for type A_{12} FMDV. Two of these nMAb's react only with either intact virus or both virus and 12S protein subunits, indicating that they recognize conformational epitopes. The remaining five nMAb's react with virus, 12S subunits, isolated VP_1, and VP_1 peptide fragments, indicating that they react with linear epitopes.

To accurately determine the location of these epitopes on the viral capsid structure, we generated neutralization-resistant variants by serial passage of virus stocks in each of the nMAb's. Plaque reduction neutralization (PRN) assays showed that the variants were no longer neutralized by the nMAb used to generate them. Each of the variants was tested by PRN assay against each of the nMAb's. The results indicated that these variants define at least four antigenic sites on the surface of type A FMDV. In addition, there may be overlapping epitopes within some of these sites. Large stocks of these variants are currently being prepared and purified for RNA isolation. Nucleotide sequencing of the structural protein region of the genome will reveal amino acid changes defining monoclonal antibody-reactive epitopes.

R16.20
MARINE CALICIVIRUSES: FINGERPRINT COMPARISONS OF GENOMIC RNA.
E.S. Berry, S.E. Poet, J.E. Gambee, M. Degner, D.E. Skilling, A.W. Smith. College of Veterinary Medicine, Oregon State University, Corvallis, Oregon, 97331, U.S.A.

Eight serotypes of San Miguel sea lion virus (SMSV) (types 4, 5, 6, 7, 8, and 13) Mink calicivirus (MCV) and Reptile calicivirus (RCV), and large and small plaque variants from SMSV-5 (5L and 5S) and SMSV-8 (8L and 8S) have been compared by genomic RNA oligonucleotide fingerprints. Each fingerprint produced was unique and easily distinguished, although similarities in the numbers and patterns of oligonucleotides were observed. All fingerprints had a heterogeneous Poly A tract and a distinct Poly C tract. The migration of the Poly C tract of each fingerprint varied with plaque size. The Poly C tract of the small plaque forming viruses (MCV, RCV, SMSV-6, SMSV-7, SMSV-5S, and SMSV-8S) migrated farther (indicating fewer oligonucleotides) than the Poly C tract of the large plaque forming viruses (SMSV-4, SMSV-5, SMSV-5L, SMSV-8, SMSV-8L, and SMSV-13). The relationship between plaque size and Poly C migration appears useful in the prediction of calicivirus pathogenic potential; the large plaque formers are more virulent than the small plaque formers.

R16.21
ANTI-IDIOTYPIC ANTIBODIES AGAINST ANTI-ENCEPHALOMYOCARDITIS VIRUS POLYCLONAL ANTIBODIES.
M. Pah Baldeh and Alfred T.H. Burness. Faculty of Medicine, Memorial University of Newfoundland, St. John's, Newfoundland, Canada.

Monoclonal anti-idiotypic antibodies (AIB) against picornaviruses reported so far are unable to recognize cellular receptors for such viruses. We have prepared polyclonal AIB against encephalomyocarditis (EMC) virus which have the ability to interact with the EMC virus red cell receptor, glycophorin. As a first step in the production of AIB, anti-EMC virus antibodies raised in New Zealand White rabbits were purified by affinity chromatography on an EMC virus-Sepharose column using enzyme-linked immunosorbent assays and hemagglutination inhibition tests to monitor column eluates and to measure the degree of purification. The purified anti-EMC virus antibodies were then injected into the same breed of rabbits to produce AIB. The AIB should mimic the physiological properties of the virus, itself. We found that the AIB bound to glycophorin immobilised on nitrocellulose and immunoprecipitated glycophorin from solubilized radiolabelled red cell membranes. Also like virus, the AIB agglutinated both human and sheep erythrocytes but not bovine nor rabbit red blood cells. These results suggest that anti-idiotypic antibodies against EMC virus have been made. Such antibodies should be extremely useful for the characterization of EMC virus receptors from a variety of cells.

R16.22
DETECTION OF HUMAN CALICIVIRUS ANTIGEN AND ANTIBODY BY ELISA.
S. Nakata, M.K. Estes and S. Chiba. Department of Virology and Epidemiology, Baylor College of Medicine, Houston, Texas USA and Department of Pediatrics, Sapporo Medical College, Sapporo, Japan.

An enzyme-linked immunosorbent assay (ELISA) was developed for detection of human calicivirus (HCV) and antibody to HCV. The ELISA was specific for HCV and as sensitive as a previously described radioimmunoassay. This ELISA was used to search for evidence of HCV infection in the USA, where HCV gastroenteritis has rarely been reported. Ninety stool samples collected from hospitalized children with diarrhea were examined; one sample was positive in the ELISA test. This sample came from a 7 1/2 month old infant who had prolonged malabsorptive diarrhea and no other detectable bacterial, parasitic, or viral cause for his illness. Typical calicivirus particles were found in this stool and these particles reacted with a guinea pig anti-HCV hyperimmune serum by immune electron microscopy. The age-related acquisition of serum antibody to HCV also was evaluated in hospitalized infants and children without gastroenteritis (age birth to 19 years) and in healthy adults. The pattern of acquisition of antibody to HCV was similar to that for group A rotaviruses. These data suggest that infections with HCV must be common and HCV can cause infantile gastroenteritis in the USA.

R16.23
EXPRESSION OF POLIOVIRUS REPLICATION GENES: ENZYMATICALLY ACTIVE POLIOVIRUS RNA POLYMERASE EXPRESSED IN CV-1 CELLS FROM A RECOMBINANT SV40 VIRUS VECTOR
M. E. Lentz, and C. D. Morrow. Dept. of Microbiology, University of Alabama at Birmingham, Birmingham, Alabama 35294

We have constructed an SV40 virus late replacement expression vector to accept a cDNA fragment of the poliovirus genome from nucleotide 5240 to the 3' end. This vector results in an in-frame fusion between the first 10 codons of the SV40 agnogene and the poliovirus coding sequences. When CV-1 cells are infected with this recombinant virus stock, bright cytoplasmic fluorescence can be seen after indirect staining with anti-polymerase antisera. Poliovirus infected cells showed similar results. Immunoprecipitation of labeled cell extracts revealed the presence of a protein which comigrates on polyacrylamide gels with polymerase ($3D^{pol}$) from poliovirus infected cells. Extracts from CV-1 cells infected with SV40 expressing $3D^{pol}$ contained detectable poly-U polymerase activity, as did extracts from poliovirus infected cells. These results indicate that a polyprotein expressed from SV40 containing 10 amino acids of SV40 agnoprotein fused to polio VP_3, protease $3C^{pro}$, and polymerase $3D^{pol}$ is properly processed to yield active polymerase. This system will allow us to more carefully analyze the steps involved in poliovirus replication.

R16.24
EXPRESSION OF POLIOVIRUS REPLICATION GENES: IN VITRO REPLICATION OF THE POLIOVIRUS GENOME BY RECOMBINANT RNA POLYMERASE EXPRESSED IN E. COLI
C.D.Morrow, M.E.Lentz and B.Warren. Dept. of Microbiology, University of Alabama at Birmingham, Birmingham, Alabama 35294

Poliovirus replication is accomplished, in part, by a viral encoded RNA dependent RNA polymerase, $3D^{pol}$. The focus of the research in this laboratory has been to define the mechanism of poliovirus replication. We have recently succeeded in the expression of enzymatically active poliovirus RNA polymerase in E. coli. We have purified the expressed polymerase (recombinant polymerase) over 35 fold from E. coli. The recombinant RNA polymerase requires either an oligo (U) primer or eukaryotic cell proteins (HeLa cell host factor (HF)) in order to replicate the poliovirus genome in vitro. In contrast to poliovirus replicase isolated from infected HeLa cells, even at the early steps of purification the replication the recombinant polymerase required HF in order to replicate the poliovirus genome. The majority of the in vitro synthesized RNA was smaller than the template RNA, but a significant fraction was of the same size or larger. We are in the process of generating mutants (both linker insertion and oligonucleotide site directed) of the $3D^{pol}$ gene which will subsequently be expressed in E. coli and analyzed for altered function in the poliovirus replication system.

R16.25

GENETIC VARIATION AND PHYLOGENETIC RELATIONSHIPS AMONG COXSACKIE B VIRUS ISOLATES AND IDENTIFICATION OF CONSERVED SEQUENCES AS TARGETS FOR PAN-ENTEROVIRUS OLIGONUCLEOTIDE PROBES. M.A. Pallansch and C.Y. Freeman. Division of Viral Diseases, Centers for Disease Control, Atlanta, GA., U.S.A.

Surveillance data from the United States show significant variation in the yearly prevalence of the six Coxsackie B (CB) serotypes. To determine if this variation correlated with variation in genotype, we sequenced the 5'-non-coding region for over 50 clinical isolates, including representatives from all six serotypes covering the period 1967-1986. These sequences showed that CB viruses fall into two major groups, the first comprising serotypes CB1, CB2, and CB6, the second group CB3, CB4, and CB5. These data also suggest CB5, which has an "epidemic" prevalence pattern, had a single genotype present during epidemic years, while the other serotypes, which have an "endemic" prevalence pattern, have multiple genotypes circulating in the same year. The sequence data also reveal significant similarities among serotypes, including three stretches of perfect homology for 18-35 bases among all the isolates. Some of these homologies are shared with sequences from other picornaviruses. Oligonucleotides were synthesized that were complementary to these sequences and used as probes to detect 57 prototype enteroviruses tested to date.

R16.26

ISOLATION OF A MONOCLONAL ANTIBODY (Rmc CB) WITH SPECIFICITY FOR THE CELLULAR RECEPTOR FOR THE GROUP B COXSACKIEVIRUSES. K-H L. Hsu, B. Alstein, R. L. Crowell. Department of Microbiology and Immunology, School of Medicine, Hahnemann University, Broad and Vine, Philadelphia, PA 19102, U.S.A.

A receptor protein (Rp-a) of 49.5 kd for the group B coxsackieviruses (CVB) was isolated in the form of a virus-receptor complex (VRC) from detergent-solubilized HeLa cells by use of CVB3 as an affinity surface (Napoles, et al, J. Virol. 1985, 55, 560). With purified VRC as immunogen, we isolated a mouse monoclonal antibody (Rmc CB) which protected HeLa cells from infection by all 6 serotypes of CVB. The Rmc CB did not protect HeLa cells from infection by polio virus, echovirus6 or coxsackievirus A18. These results confirmed the biological specificity of the antibody. The Rmc CB also protected BGM cells, a monkey kidney cell line, from infection by CVB. Thus, this Rmc CB shows a different receptor epitope specificity than one obtained previously (Crowell, et al, J. Virol. 1986, 57, 438) which blocked receptors for only CB1, CB3 and CB5 and echovirus6. Furthermore, the Rmc CB immunoprecipitated two HeLa cell proteins of 44kd and approximately 150-200 kd. The relationship between these proteins and the Rp-a remains to be determined. It is possible that more than one protein comprises a receptor complex for binding CB viruses to the HeLa cell plasma membrane. (Supported by U.S. Public Health Service grant AI-03771 from NIAID).

R16.27

CHARACTERIZATION OF THE VIRUS SPECIFIC RNAS IN CALICIVIRUS INFECTED CELLS. J.D. Neill and W.L. Mengeling. USDA-ARS, National Animal Disease Center, Ames, Iowa 50010 USA.

The caliciviruses are a family of small, non-enveloped viruses that have a plus-stranded, polyadenylated RNA genome. Members of this family include feline calicivirus, San Miguel sea lion virus and vesicular exanthema virus of swine. The host range and biophysical characteristics have been determined but little is known of the molecular biology of these viruses.

To elucidate the number and temporal appearance of viral specific RNAs in infected cells, total cellular RNA from calicivirus-infected cells was analyzed by northern blotting. The RNA was size fractionated on 1.2% agarose-formaldehyde denaturing gels, blotted to nitrocellulose and probed with ^{32}P-labeled cloned viral cDNA probes. This analysis showed that there is a set of virus-specific RNAs ranging in size from approximately 700 bases to the full length viral genome of approximately 8000 bases. By analysis of RNA isolated at hourly intervals post-infection, it was determined that these virus-specific RNAs appear at 2 to 3 hours post-infection and continue to accumulate through the infection cycle.

R16.28

SITE-SPECIFIC MUTAGENESIS OF THE GENOME-LINKED PROTEIN (VPg) OF POLIOVIRUS. R. J. Kuhn, H. Tada, and E. Wimmer. Department of Microbiology, State University of New York at Stony Brook, Stony Brook N.Y. USA.

The 5' terminus of all poliovirus-specific RNA found in infected cells is blocked by a small polypeptide termed VPg. Although the function of VPg is unknown, it has been postulated and recent data supports, a model in which VPg functions as a primer for the primer-dependent RNA polymerase $3D^{pol}$. A genetic analysis of VPg has been carried out to determine the specific amino acid requirements of VPg and how they are related to its function(s)

Mutations were constructed using a synthetic mutagenesis cartridge which inserted unique restriction sites in the region of the genome coding for VPg. Replacement of wild-type sequences within the restriction sites was accomplished by the insertion of complementary synthetic oligonucleotide duplexes. For example, mutants affecting the tyrosine residue which forms the linkage to the RNA were unable to generate infectious virus, suggesting the position of the tyrosine residue is critical to the function of VPg. In contrast, a series of viruses have been constructed which insert methionine residues in place of the wild-type amino acids. These viruses have normal phenotypes and exhibit wild-type growth kinetics. They will be useful in following the fate of VPg under both *in vivo* and *in vitro* conditions.

R16.29

ENCEPHALOMYOCARDITIS VIRAL INFECTION IN HUMAN ERYTHROLEUKEMIC CELLS (K562). P. Wanda and M. Hart, Department of Biological Sciences, Southern Illinois University, Edwardsville, IL 62026, USA. Glycophorin A, isolated from human erythrocytes, has previously been identified as receptor substance for encephalomyocarditis (EMC) virus (Allaway, G. P. and Bruness, A.T.H., J. Virology 59:768). We report here, the use of a human erythroblastoid cell line, K562, as an ideal human host cell system for EMC virus production. Unique to this system is the expression of glycophorin A and the induction of erythroid differentiation (hemoglobin production) after addition of 45 μM bovine hemin. Hemoglobin production was determined by benzidine staining of cells and reached maximum levels (80%) in 3-4 days following induction. EMC virus was added to induced and non-induced cells and at 24 hours post-infection (p.i.) both cultures showed characteristic cytopathic effects. Between 48-54 hours p.i., the induced cultures showed complete cell destruction and plaquing in Vero cells showed maximum virus production. A 10-fold reduction in virus titer post-54 hours is due to thermal inactivation. Induction of erythroid differentiation did not enhance viral adsorption as determined by infectious cell center assays. Viral replication appeared not to interfere with hemoglobin synthesis.

R16.30

CELL-FREE EXPRESSION OF COXSACKIEVIRUS 3C PROTEASE FROM A c-DNA CLONE WHICH CONTAINS THE TRANSLATIONAL INITIATION SIGNAL OF AN INSECT VIRUS.
B. Dasmahapatra, E. Rozhon, J. Schwartz. Dept. of Antiviral Chemotherapy, Schering Corp., Bloomfield, NJ 07003 U.S.A.

The genome of coxsackievirus (CVB_3), like that of other picornaviruses, is translated into a large polypeptide which is proteolytically processed by virally encoded proteases to yield mature viral proteins. Novel plasmid DNAs containing the 5' noncoding region of the black beetle virus (BBV) genome linked to the CVB_3 genomic segment coding for part of the large polypeptide (including the 3C protease) have been constructed and transcribed into RNA. Like BBV RNA, the chimeric RNA containing ribosome binding sequences and the initiator AUG of BBV fused to the CVB_3 coding region, directed efficient synthesis of CVB_3 protein in a cell-free system. The plasmid pC1B1 produced a selfcleaving CVB_3 protein precursor which contained biologically active $3C^3$ protease. Insertion of a DNA linker within the 3C coding region in pC1B1 abolished endogenous selfcleavage activity. Using BBV translational initiation signal we have expressed CVB_3 capsid protein precursor (P_1), and demonstrate that the *in vitro* synthesized 3C protease cleaves P_1 capsid precursor into VP_0, VP_3 and VP_1. These experiments show that functional CVB_3 3C protease can be produced in a cell-free system using the insect virus (BBV) translational initiation signal, which may also be used for the cell free expression of any gene segment.

R16.31

EXPRESSION OF FOOT-AND-MOUTH DISEASE VIRUS PROTEINS IN A CELL-FREE SYSTEM AND IN MAMMALIAN CELLS. D.Moore, V.Vakharia, M.Devaney, M.Grubman, J.Dunn, and T.Fuerst. Plum Island Animal Disease Ctr, ARS/USDA, Greenport, NY, Dept Biology, Brookhaven National Lab, Upton, NY, and NIAID, NIH, Bethesda, MD.

Foot-and-mouth disease virus (FMDV) genes are expressed as a large polyprotein which is rapidly processed into mature capsid proteins and nonstructural proteins. Cell-free translation of FMDV RNA in a rabbit reticulocyte system produces functional proteins, including a viral protease, 3C, which plays a major role in the processing of the precursor proteins. To study the proteolytic processing events of FMDV, cDNA segments encoding various regions of the FMDV genome were cloned into a plasmid containing a T7 promoter region. RNA transcripts were prepared using T7 RNA polymerase and then translated in rabbit reticulocyte lysates. The expressed FMDV proteins were identified by immunoprecipitation with specific antisera and analyzed by gel electrophoresis. The results of proteolytic processing of FMDV will be presented. FMDV proteins were expressed in vivo in mammalian cells using a transient-expression system utilizing infection with a recombinant vaccinia virus expressing T7 RNA polymerase and transfected with plasmids containing FMDV cDNA segments under control of the T7 promoter. The harvested cells were analyzed for the expression and processing of FMDV proteins by western blotting. The results of in vivo FMDV protein expression will be presented.

R16.32

ANTIGENIC VIRAL VARIANTS WITHIN ONE OUTBREAK OF FOOT AND MOUTH DISEASE. M.G.Mateu, E.Rocha, O.Vicente, F.Vayreda, C.Navalpotro[2], D.Andreu[2], E.Pedroso[2], E.Giralt[2], L.Enjuanes & E.Domingo, Centro de Biología Molecular (CSIC-UAM), Universidad Autónoma, Canto Blanco, 28049 Madrid, Spain and 2: Departament de Química Orgànica, Universitat de Barcelona, 08028 Barcelona, Spain.

The antigenic variation among 14 isolates of foot and mouth disease virus (FMDV) of type C_1 from one disease outbreak has been evaluated using a set of monoclonal antibodies (MAbs) produced against one of the viral isolates (C-S8c1). Three MAbs that reacted with capsid protein VP3 and one with VP2 were non-neutralizing and showed no differences of reactivity with the 14 isolates by western blotting (WB) and by immunodotting (ID) with complete virions. Seven neutralizing MAbs were directed to at least two epitopes because they displayed two distinct patterns of reactivity. These epitopes were mapped within aminoacids 136-156 of VP1 by ID with synthetic peptides. One of these MAbs reacted equally with the 14 isolates but the other six distinguished the isolates in two groups. The differences of reactivity were 10^2-10^3 fold in WB and 10-10^2 in both ID and neutralizing activity. Thus, cocirculation of viruses with a distinct antigenic structure at a critical epitope occurs during a FMD outbreak.

R16.33

VIRUS AND CELL HETEROGENEITY DURING A PERSISTENT INFECTION WITH FOOT-AND-MOUTH DISEASE VIRUS IN VITRO. J.C. de la Torre, E. Martínez-Salas, J. Díez, F. Gebauer and E. Domingo, Centro de Biología Molecular (CSIC-UAM), Universidad Autónoma, Canto Blanco, 28049 Madrid, Spain.

Foot-and-mouth disease virus (FMDV) variants have been isolated from BHK-21 cell cultures persistently infected with FMDV. Sequential mutations fixed in viral RNA as well as genetic heterogeneity within viruses isolated from cells at one passage have been identified by RNA sequencing. Variants highly cytolytic for BHK-21 cells are selected in the course of persistence. Analysis of 248 individual cell clones derived from the carrier cultures at early and late passages has revealed heterogeneity with regard to cellular morphology and sensitivity to FMDV. At least seven distinct cell phenotypes were present, and they are due to stable genetic changes of the cells. Thus, in a persistent infection initiated by plaque-purified FMDV and by a cloned BHK-21 cell population, variant cells with increased resistance to FMDV are selected. We suggest that the interaction of heterogeneous FMDV with an heterogeneous host cell population, contributes to the maintenance of the persistent infection.

R16.34

pH-DEPENDENT UNCOATING OF POLIOVIRUS IN ACIDIC ORGANELLES - INHIBITION STUDIES WITH DISOXARIL (WIN 51711). H.Zeichhardt*, M.J.Otto+, M.A.McKinlay+, P.Willingmann* & K.-U.Habermehl*. *Institute for Clinical and Experimental Virology, Free University of Berlin, Germany; +Sterling Winthrop Research Institute, Rensselaer, N.Y., USA.

Disoxaril (WIN 51711, 5-[7-[4(4, 5-dihydro-2-oxazolyl)phenoxy]hepty1]-3-methylisoxazole) inhibits poliovirus replication by stabilizing the virus capsid which results in blocking the pH-dependent uncoating of poliovirus in the acidic endosomes and/or lysosomes: Polioviruses types 1 and 2 entered HeLa cells by receptor-mediated endocytosis via coated pits and coated vesicles into endosomes irrespectively of the presence and absence of the compound as shown by electron microscopy. Measurement of viral RNA synthesis revealed that disoxaril inhibited the arrival of viral RNA in the cytoplasm for new RNA synthesis completely only when the virus was preincubated with disoxaril (0.3 µg/ml for poliovirus type 1, 0.03 µg/ml for type 2). Simultaneous addition of the compound and virus resulted in reduced inhibition of viral RNA synthesis. The inhibitory effect of the compound could be partially reversed up to 25 min p.i. if the compound was eluted from the cells. At that time after infection the virus was located in endosomes.

R16.35

THE STRUCTURE OF COWPEA MOSAIC VIRUS AT 3.0 Å RESOLUTION. C. Stauffacher, R. Usha, T. Schmidt, M. Harrington, J. E. Johnson. Department of Biological Sciences, Purdue University, West Lafayette, IN, U.S.A.

Cowpea mosaic virus (CpMV) is a multicomponent RNA plant virus which shows physical and biological similarity to the animal picornaviruses. Further parallels between the capsid proteins of these two viruses have been proposed on the basis of the gene order of their RNA's. The 3.0 Å x-ray crystallographic structure of CpMV shows these similarities as expressed in the capsid structure. The T=1 capsid of the virus contains 60 copies each of a large (42kD) and a small (24kD) protein. These two proteins fold into three β-barrel domains which mimic in many details the three independent β-barrel domains of the major proteins in the picornavirus capsid. The arrangement of the domains reflects the alignment of the gene order, with differences in the surface loop structure producing the characteristic shape of CpMV as seen in the electron microscope. Since both comoviruses and picornaviruses produce polyproteins which are later processed in the capsid assembly, CpMV can be considered a model for processing where two of the β-barrel domains remain uncleaved in the native capsid. Such close parallels between the capsid structures as seen by x-ray crystallography suggest strong evolutionary relationships between the comoviruses and the picornaviruses.

R16.36

THE COMPLETE NUCLEOTIDE SEQUENCE OF A BOVINE ENTEROVIRUS. E.M. Hoey, J.A.P. Earle, R.A. Skuce, S.J. Martin. Dept. of Biochemistry, Q.U.B., Medical Biology Centre, 97 Lisburn Road, Belfast, N. Ireland.

Overlapping cDNA clones spanning the entire genome of a bovine enterovirus (VG-5-27) have been produced in E.coli and used to determine the complete nucleotide sequence. The genome is 7,414 nucleotides long and consists of a 5' non-coding region of 818 nucleotides, a long open reading frame of 6525 nucleotides and a stretch of 71 nucleotides at the 3' end before the poly 'A' tract. The genome organisation is similar to that of the polioviruses and rhinovirus 14 and considerable homology exists between them at the amino acid level. This homology is greatest for the non-structural proteins and least for the structural proteins. Alignments have allowed us to predict most of the polyprotein cleavage sites which are predominantly between gln/gly pairs.

We have spliced together several of the clones to create a plasmid containing the full length genome. This plasmid has proved to be infectious in BHK cells.

R16.37

EVIDENCE LINKING COXSACKIE B VIRUSES WITH PERSISTENT INFECTIONS IN PATIENTS WITH CHRONIC CARDIAC DISEASE.

P. Muir, A.J. Easton, T.G. Wreghitt, S. Stewart, P.G. Stovin, T.A.H. English and J.E. Banatvala (UMDS, St. Thomas' Campus/University of Warwick/PHLS, Microbiology and Papworth Hospital, Cambridge.

Employing an M-antibody capture technique, Coxsackie B virus (CBV) specific IgM responses were detected in six patients with recurrent pericarditis. Responses were present during the entire course of the disease, which varied from 14 months to 5 years. In contrast, patients with uncomplicated acute CBV infection had responses which rarely persisted for longer than 3-6 months. CBV-specific IgM responses were also detected in 5/12 (41.7%) patients with dilated cardiomyopathy. These responses may persist after cardiac transplantation, perhaps because the recipients' atria are not removed. Preliminary studies show that some patients have CBV sequences in myocardial tissue. Although these studies suggest that CBV induces persistent infection in man, the mechanism by which cardiac damage occurs remains to be established. Results of investigations correlating serological responses and CBV RNA in myocardial tissue with HLA DR phenotypes and cardiac autoantibodies will be presented in order to determine whether cardiac damage is mediated by an autoimmune mechanism.

R16.38

ANTI-POLIOVIRUS MONOCLONAL ANTIBODIES : CORRELATION OF ANTIGENIC SITE AND RECOGNITION OF 14 S SUBVIRAL PARTICLES

R. Vrijsen, B. Rombaut and A. Boeyé. Department of Microbiology and Hygiene, Vrije Universiteit Brussel, Laarbeeklaan 103, B-1090 Brussels (Belgium).

Monoclonal antibodies that neutralize poliovirus can be classified as N1- or N2-specific according to whether they do or do not immunoprecipitate 14 S subviral particles. N2 epitopes are products of maturation since they are expressed on procapsids and mature virions only. It remained to be seen whether the N1 and N2 epitopes belonged to distinct antigenic sites as defined by cross-resistance of mutants to neutralization.

This was studied using mutants of Mahoney (type 1) poliovirus selected for their resistance to neutralization by antibodies of N1 and N2 specificity. The M1 mutant resistant to an N1-specific antibody was neutralized by none of 4 N1-, and by all of 10 N2-specific antibodies. Conversely, the M2 mutant resistant to an N2-specific antibody was neutralized by all N1- but not by any of the N2-specific antibodies.

It is concluded that the M1 and M2 mutants exhibited cross-resistance to all neutralizing antibodies of the same (N1 or N2) specificity as the selecting antibody, and only to those. The N1 and N2 epitopes therefore define separate antigenic sites.

R16.39

DIFFERENTIAL INHIBITION OF POLIOVIRUS STRAINS BY L-PHENYLALANINE : A POSSIBLE GENETIC MARKER.

V. Kadam and S. Paranjape, National Institute of Virology, 20-A Dr Ambedkar Road, PUNE 411 001, India.

15 strains of the 3 types of Poliovirus (3 wild type and 12 vaccine strains) were screened for their L-phenylalanine requirements by comparing the efficiency of plating and plaque morphology on monkey kidney monolayers in the absence and presence of different concentrations of the amino acid. The 3 wild type strains required 32-64 mg/lit of L-phenylalanine, 10 vaccine strains were inhibited (70-86% depending on the strain) by as little as 1.6 mg/lit of the amino acid and the growth of 2 vaccine strains was independent of the concentration of the amino acid.

Infectious genomic RNA isolated from either phenylalanine sensitive or dependent strains retained the particular phenylalanine character of the parental viruses.

The phenomenon showed the following characteristics: 1) The D isomer of the amino acid was not inhibitory but it could not reverse the inhibition caused by L-isomer. 2) Inhibition could not be attributed to the chelating activity of L-phenylalanine. 3) The amino acid restricted the virus replication at 37°C (and not at 31°C) implicating that the phenomenon is temperature dependent. 4) The adsorption of the virus was not affected but the eclipse phase was prolonged by 2.5 hours. 5) Viral RNA and protein syntheses were not affected.

R16.40

POLIOVIRUS TYPE 1/TYPE 3 ANTIGENIC HYBRID VIRUS: CHARACTERIZATION AND PROPERTIES.

Michael G. Murray, Richard J. Kuhn and Eckard Wimmer, Dept. of Microbiology, State University of New York at Stony Brook, Stony Brook, U.S.A.

It is known that almost all murine monoclonal antibodies (mcAbs) elicited by poliovirus type 3 (PV-3) react with a single major antigenic site (site 1) located on capsid protein VP1. In contrast, mcAbs elicited by poliovirus type 1 (PV-1) react with sites distinct from site 1 located on capsid proteins VP2 and VP3. In order to study the relationship between structure and antigenicity a PV-1/PV-3 hybrid virus was constructed.

Using site specific mutagenesis, synthetic oligonucleotides and a full length infectious cDNA clone of PV-1, a plasmid was constructed which replaces site 1 of PV-1 with the sequence corresponding to site 1 of PV-3. The recombinant DNA was transcribed in vitro and transcripts were transfected into mammalian cells. Resulting virus was determined to have the expected PV-1/PV-3 hybrid sequence. PV-3 specific antiserum as well as PV-1 specific antiserum were capable of neutralizing the hybrid virus. In addition it was found that the primate immune system recognizes site 1 of PV-3 in a natural immune response.

R16.41

OLIGONUCLEOTIDE HYBRIDIZATION REVEALS UNEXPECTED INTERTYPIC SEQUENCE RELATIONSHIPS IN THE 5'-NONCODING REGION OF POLIOVIRUS GENOME.

T.Hovi, T.Pöyry and L.Kinnunen, Enterovirus Laboratory, Department of Virology, National Public Health Institute, Helsinki, Finland.

The 5'-noncoding part of the genome of the wild-type 3 poliovirus strain associated with the recent outbreak of poliomyelitis in Finland (P3/Finland/23127/84) contains regions identical to those in the type 1/Mahoney strain and with only 45% nucleotide homology with the corresponding region in the type 3/Leon or Sabin strains (Hughes et al, J. Gen. Virol. 67:2097, 1986). By using two oligo(16)-nucleotide probes synthesized according to the sequences of the P1/Mahoney and P3/Leon strains, respectively, we showed that the P1/Mahoney-like sequence is shared by all the 70 tested type 3 strains of the Finnish outbreak, but is relatively rare among independent isolates from the rest of the world.

Since this sequence is stable within an outbreak and rare among poliovirus isolates in general, the probe hybridizing to this sequence may be a useful tool in searching for the origin of the outbreak. Background for the fact that this sequence exists but is rare among both type 1 and type 3 strains can only be speculated at the moment. Intertypic recombination and an important regulatory function with a strong selection power are among the theoretical alternatives.

R16.42

PROPERTIES IN VIVO AND IN VITRO OF "CAPPED" OR DELETED GENOMES OF POLIOVIRUS TYPE 1 GENERATED IN VITRO BY T7 RNA POLYMERASE.

Jonathan Bradley*, G. Drugeon**, K. Kean*, E. Wimmer***, M. Girard*, *Institut Pasteur, Paris, France. **Institut Jacques Monod, Paris, France. ***Dept. of Microbiology, State University of New York at Stony Brook, Stony Brook, U.S.A.

Using an efficient T7 RNA polymerase transcription system producing highly infectious poliovirus RNA from suitable cDNA clones (v. d. Werf, Bradley, J., Wimmer, E., Studier, F.W. and Dunn, J.J., 1986, PNAS 83:2330) we have produced "capped" poliovirus RNA and studied its ability to program rabbit reticulocyte lysates (RRL) in vitro. "Capping" appeared to have no stimulating effect in translation when compared to uncapped virion or transcript RNA. Deletion mutants of poliovirus have been engineered by removing part of the P1 (capsid) region in frame and out of frame. The deleted RNA produced the expected proteins upon in vitro translation in RRL. The in frame deletion was found viable upon co-transfection with a traceable helper virus while the out of frame deletion proved to be non viable. The ability to make infectious deletion derivatives of poliovirus should allow a search for cis-acting viral elements.

R16.43

CHEMICAL MODIFICATION OF THE LEADER PROTEIN (Lb) OF FOOT AND MOUTH DISEASE VIRUS. D.V. Sangar, D.J. Rowlands, R.P. Clark, E. Westaway, A. Syred, A.R. Carroll, J.F.E. Newman, B.E. Clarke. Wellcome Biotechnology, Pirbright, Surrey, UK.

The translation product of FMDV RNA is a polyprotein which consists of a leader protein followed by P1, P2 and P3 domains. Translation initiates from either of two in frame AUG codons both in vitro and in vivo, resulting in the production of two alternative forms of the leader protein, one of mol wt 20k (Lab) and one of 16k (Lb). The relative usage of the two codons depends on the surrounding nucleotide sequences in different strains of the virus and on the conditions for translation. In vitro, at 2mM Mg^{2+} concentration, Lb is the major product but raising the Mg^{2+} concentration to 4mM favours the Lab initiation codon.

Extended incubation of cell free lysates primed with genomic RNA or SP6 transcribed subgenomic RNAs, under conditions which favour the production of the Lb protein, results in the production of a new protein of apparent mol wt 22k (P22). This protein is not observed when Lab is the major leader protein synthesised. Protein P22 appears to be a form of Lb which is modified after translation. The experimental data supporting this conclusion and suggestions concerning the nature of the modification will be presented and discussed.

R16.44

AN INDUCIBLE AMBER SUPPRESSOR MAMMALIAN HOST CELL SYSTEM. J.M. Sadivy, J.P. Capone*, U.L. Rajbhandary and P.A. Sharp, Center for Cancer Research & Dpt. of Biology, MIT, Cambridge, MA and *McMaster University, Hamilton, Canada

We describe a general protocol for controlled gene amplification, which allows conditional expression of high levels of amber suppressor activity in monkey cells, and we demonstrate its use in the genetic analysis of animal viruses by the generation and propagation of the first nonsense mutant of poliovirus. A human amber suppressor $tRNA^{Ser}$ gene was covalently linked to the SV40 origin of replication and co-transfected into monkey cells along with a temperature-sensitive mutant of the SV40 large T antigen gene, resulting in the stable integration of a few copies per cell of each plasmid at the non-permissive temperature. Upon shift-down to the permissive temperature, DNA replication was initiated at the SV40 origin, resulting in the amplification of the origin and the linked Sup^{+} tRNA gene to levels of 2,000-4,000 copies per cell. This amplification, as episomal DNA, was complete within 48 hrs, occurred in the majority of the cells, and resulted in 50-70% suppression efficiencies of amber codons. A mutant of poliovirus, in which a serine at position 28 in the replicase gene was converted to amber, could be efficiently propagated on the suppressor cell lines. The mutant virus reverted to wild type only by an A to C transversion from amber (UAG) to serine (UCG), indicating a requirement for an aliphatic hydroxyl group at this position in the ensyme. The availability of this mutation permitted the first analysis of the mutational frequency at a defined single locus in poliovirus, which was surprisingly low, approximately 2.5×10^{-6}.

R17.1

STRUCTURAL HETEROGENEITY OF HBsAg FORMS. M. Peeso M. Tănase, N. Cajal. Immunochemistry Unit, "S.Nicolau" Institute of Virology, Bucharest, Romania.

The separation of HBsAg forms from high titer plasma obtained from "healthy" carriers, by mild procedures /mainly gelfiltration/ results in preparations which main activity is not adsorbed by Con A-Sepharose. Serologically active fractions further differ with respect to their sensitivities to nonionic detergents and/or pepsin, the main Con A unadsorbed fraction being unusually sensitive. Repeated combined treatment reveals diverging patterns, when the recoveries of serologically active and of bulk proteins are compared: both indices of the Con A unbound fraction fall at fairly equal rates, while the Con A bound, serologically active material becomes much more pepsin resistant than the bulk protein of the same fraction. Different titration slopes and specific titers (per mg) are observed in RIA or EIA, depending on the origin of the labelled anti-HBs.

It is hypothesized that the phenomenon reflects variations in the relative presence of the pre-S and S proteins, and of host serum albumin.

R17.2

A LONGITUDINAL STUDY OF HEPATITIS B IN A UNIVERSITY
Liu Feili et al, Department of Epidemiology, Shanghai Medical University People's Republic of China

A group of 843 students enrolled in a University in the fall of 1983 were tested by ELISA method, among which 426 were males and 417 were females. The total HBV infection rate was 59.79%, HBsAg positive rate 10.44%, anti-HBs 19.30% and anti-HBc 30.01%. The total HBV infection rates were 62.20% and 57.97%, and the HBsAg positive rates were 12.68% and 8.15% for male and female respectively. The HBsAg positive rate for males exceeded slightly over that for female. We re-examined 753 students one year later, 96% of the 451 HBV infected students still persisted positive whereas 3.99% converted to negative, of the 302 susceptibles to hepatitis B positive seroconversion rate was 2.32%. The potential transmission route of hepatitis B in the University was discussed.

R17.3

HEPATITIS B $_s$Ag IN CHRONIC ASYMPTOMATIC HEPATITIS B SURFACE ANTIGEN CARRIERS AND IN PRIMARY HEPATOCELLULAR CARCINOMA PATIENTS. S. A. Tawana
Department of Medical Microbiology, Faculty of Medicine, University of Zimbabwe, Harare, Zimbabwe.

A total of 246 HB$_s$Ag - positive subjects were tested for the presence of Hepatitis B$_s$ antigen, and total anti-delta IgG. Of the 246, 174 were HB$_s$Ag - positive asymptomatic chronic carriers and 72 were Primary hepatocellular carcinoma patients attending the two teaching hospitals, i.e. Parirenyatwa and Harare Central Hospitals.

All sera were examined by the Enzyme-linked immunosorbent assay (ELISA), and results were read spectrophotometrically at a wavelength of 492nm. 82 (33.3%) were HB$_s$Ag - positive. Of the 246, 56 (39.4%) males and 26 (25%) females had HB$_s$Ag present in the serum. However, the prevalence of HB$_s$Ag from only the chronic asymptomatic HB$_s$Ag - positive carriers in males and females was 17 out of 103 (16.5%) and 12 out of 71 (16.9%) respectively. Thus with an overall prevalence of 31 (17.8%) from the asymptomatic group. Consequantly, the prevalence of HB$_s$Ag from the Primary Hepatocellular carcinoma (PHC) group was 32 out of 72 (44.4%). The rate of positives from the males and females of the PHC group was 46.2% and 42.4% respectively. Total anti-Delta IgG was not detected in the 246 examined sera.

R17.4

INTEGRATION OF HBV DNA INTO LIVER TISSUES OF CHRONIC HEPATITIS PATIENTS IN EARLY CHILDHOOD. T. Morishima, Y. Goto, H. Nagai, K. Kuzushima, H. Yamaguchi, M. Kobayashi, K. Yaginuma and K. Koike. Department of Pediatrics, School of Medicine, Nagoya University, Nagoya and The Department of Gene Research, Cancer Institute, Tokyo, Japan.

In order to determine when the HBV DNA is integrated into chromosomal DNA of liver tissue, biopsy samples were obtained from 5 HBV chronic hepatitis patients between 4 and 9 year-old. All of them were infected with HBV from their mothers perinatally. Purified DNA was digested with the restriction enzyme, Hind III, and then subjected to agarose gel electrophoresis and analyzed by the Southern blot hybridization.

Diffuse hybridization from 7.6 to 20 Kb in size was observed in all 5 samples following Hind III digestion, indicating that HBV DNA was integrated into liver tissues of the patients, and that the populations of cells containing HBV DNA are heterogeneous. In contrast, no hybridization was observed in the autopsy liver obtained from the neonate who died on day to after birth at 25 weeks of gestational age. (His mother was a asymptomatic carrier with HBs and HBeAg.) Thus, HBV DNA can be integrated into chromosomal DNA of liver in most of children after perinatal infection of the virus, but not in the fetus. Follow up care should be made available for HBV carrier as pre-cancerous stage.

R17.5

HEPATITIS B VIRUS INFECTION OF CORD BLOOD LEUKOCYTES.
H. Shen, K. Choo, T. Wu, H. Ng, S. Han. Department of Medical Research, Veterans General Hospital, Taipei

The presence of hepatitis B virus (HBV) DNA in the serum and leukocytes obtained from the peripheral blood of twenty-four mothers and from the cord blood of their newborns was determined by hybridization procedures. No HBV DNA was detected in the serum and leukocytes of six HBsAg-, HBeAg-negative and two HBsAg-positive, HBeAg-negative mothers and their newborn infants. Among the sixteen HBeAg-positive carrier mothers, HBV DNA was found in thirteen cases (81%) in the serum and two cases (12%) in the leukocytes. Though the viral DNA was not present in sera, it was detected in two of the sixteen cord blood leukocyte samples. In follow-up studies, these two infants were found to be failures in HBsAg seroconversion up to fifteen months of age and they became carriers with elevated serum alanine aminotransferase levels. Our results suggest that HBV may be transmitted vertically and such in utero infection may have resulted in immune tolerance leading to a carrier state. (Supported in part by a grant NSC-75-0610-B075-23 from National Science Council of the Republic of China).

R17.6

HEPATIC AND SPLENIC SPECIFICITY OF WOODCHUCK HEPATITIS SURFACE ANTIGEN ATTACHMENTS. T.I. Michalak, Faculty of Medicine, Memorial University of Newfoundland, St. John's, NF, Canada.

Woodchuck hepatitis virus (WHV) closely resembles hepatitis B virus (HBV) and is presently considered as strictly hepatotrophic. Several lines of evidence indicate the possibility of the extrahepatic replication of HBV. In an attempt to recognize the extrahepatic sites of WHV attachments, radiolabeled purified WHV surface antigen (WHsAg) was incubated with slices of homologous and heterologous liver, spleen, pancreas, heart, and kidney tissues. The bindings were performed at 22°C in protein free medium over both time and ^{125}I-WHsAg concentration. The results revealed saturable adsorption WHsAg to woodchuck liver and spleen over both tested parameters. At the saturation level, the spleen bound WHsAg at a ratio not less than 55% of homologous liver. The bindings to both organs were significantly diminished by preincubation of the slices with an excess of unlabeled WHsAg, but were unblockable by woodchuck polymerized serum albumin. WHsAg was unable to specifically interact with other woodchuck organs and with human, rabbit, guinea pig and rat tissues. The exception was found for human normal liver, that bound WHsAg at about 60% of woodchuck liver. These results demonstrate hepatic, as well as splenic specificity of WHsAg binding. Thus, they implicate the possibility of WHV entry into splenic cells and the virus propagation in the host spleen. These data also suggest a partial identity of WHsAg binding sites within woodchuck and human livers. (Supported by MRC Canada grant MA-9256).

R17.7

DELAYED TYPE HYPERSENSITIVITY SKIN REACTION TO HEPATITIS B SURFACE ANTIGEN IN PATIENTS WITH TYPE B ACUTE AND CHRONIC HEPATITIS.

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The First Department of Medicine*, Faculty of Medicine, Kyushu University, The Department of Pathology**, School of Medicine, Fukuoka University, and Division of Internal Medicine, Fukuoka Teishin Hospital***, Fukuoka, Japan.

Delayed type hypersensitivity (DTH) skin reaction to HBsAg was studied in patients with type B acute and chronic hepatitis. HBsAg preparations containing 50 µg/ml of antigen, both with and without alum, elicited positive DTH skin reactions in anti-HBs+ persons. Irrespective of the presence of HBeAg and anti-HBe, patients with the chronic type B hepatitis responded to PPD and SK/SD antigens, whereas there was no DTH skin reactivity to HBsAg. In contrast, while the patients with acute type B hepatitis did not exhibit a specific DTH reaction to HBsAg in the acute period, DTH skin reactivity to HBsAg occurred in the convalescent phase of the disease, preceding the appearance of anti-HBs. Immunohistopathologic study of biopsied sample of livers from patients with type B acute hepatitis revealed accumulation of T4 lymphocytes in the portal/periportal area. Infiltrations of T8 cells and proliferation of Kuppfer cells were present in the lobule. B lymphocytes were absent in all areas of the liver. Therefore, both cytotoxic T cells and the DTH reaction may be required for recovery from the type B acute viral hepatitis.

R17.8

PROPERTIES AND STABILITY OF THE PRE-S REGION OF HEPATITIS B VIRUS (HBV)

S.-H. Chen, S.E. Brown, M.W. Steward and C.R. Howard, Department of Medical Microbiology, London School of Hygiene and Tropical Medicine, London, UK.

Pre-S antigens and antibodies have been examined using monoclonal antibodies and synthetic peptides. Conditions have been established for the stabilization of pre-S(2) antigens on HBsAg particles. Formalin treatment was found to stabilize major pre-S(2) antigens. Analysis of pre-S(2) +ve HBsAg particles showed that (a) polymerized albumin-binding activity is associated with polypeptides bearing pre-S(2) determinants only and not those containing additional pre-S(1) sequences, and (b) HBsAg particles purified from HBeAg-positive plasma can be separated into two populations on the basis of albumin-binding activity. Animal pre-S antibodies were obtained with a peptide containing residues LQDPRVRGLY within the pre-S(2) region and a peptide representing residues 92 to 107 within pre-S(1). Human convalescent sera bound to both peptides and additionally a pre-S(1) peptide representing residues 26 to 34. Pre-S(1) antibodies develop during acute hepatitis B, preceding an anti-HBs response.

R17.9

PERINATAL TRANSMISSION OF HEPATITIS B VIRUS: PREDICTIVE VALUE OF HBV MARKERS.

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(1) College of Medicine, King Saud University, Riyadh, Saudi Arabia; (2) Hopital Hôtel-Dieu de Montréal, Faculty of Medicine, Université de Montréal, Montreal, Qué., Canada; (3) Center for Disease Control, Atlanta, Ga., U.S.A.

The study of the distribution of various markers of hepatitis B virus in carrier pregnant mothers in Saudi Arabia show that 11% of them were HBeAg-positive compared to 82% who were anti-HBeAg positive and 6% who were negative for both HBeAg and anti-HBe. All carrier mothers who were HBeAg-positive were also positive for HBV-DNA.

The carrier mothers who were negative for HBeAg and anti-HBe were also negative for HBV-DNA and IgM anti-HBc. It seems, from our study, that HBV-DNA is a better predictor of perinatal transmission than the HBeAg/anti-HBe system. The rate of perinatal transmission of HBV infection was 100% when the carrier mother was HBV-DNA positive irrespective of HBeAg/anti-HBe status.

R17.10

PERINATAL TRANSMISSION OF HEPATITIS B IN IRAQ

D.A.N. Mohammed, T.S. Al-Hadithi, S.A. Chaidi and A.R. Omer. Department of Community Medicine, College of Medicine, Baghdad University, Baghdad, Iraq.

The carrier rate of HBsAg among 1100 Iraqi pregnant women screened antenatally was 3.7%. The total prevalence of HBV serological markers was 58.5%. Of these carrier mothers, 5% were HBeAg positive and 36.6% were anti-HBe positive.

A follow up study of 30 HBsAg carrier mothers and their newborn infants over a period of 6 months revealed HBsAg positivity in 6 (20%) of infants, which remained positive in 4 (13.3%) infants at the age of 6 months.

There was a strong association between maternal HBsAg positivity and subsequent antigenaemia in the infants, as both infants born to HBsAg positive mothers became HBsAg positive while only 4 (14.3%) out of 28 infants born to HBsAg negative mothers became HBsAg positive.

The overall findings indicate that the rate of perinatal transmission of HBV is low in Iraq.

R17.11

BIOLOGICAL AND BIOCHEMICAL CHARACTERISTICS OF HIGHLY PATHOGENIC HUMAN ISOLATE OF WEST NILE VIRUS IN THE CENTRAL ARICAN REPUBLIC

C.C. Mathiot, J.P. Gonzalez, and A.J. Georges, Pasteur Institute of Bangui, Central African Republic; Institut Français de Recherche Scientifique pour le Développement en Coopération, ORSTOM Bangui, CAR.

Since 1973, five human strains of West Nile Virus (WNV) have been isolated in the Central African Republic (CAR). In 1983 an outbreak of WNV occured in Bangui, the capital of CAR. Four strains were isolated from patients with acute hepatitis, of whom two died while two impaired. Clinical and/or histological findings showed that the liver was a major target organ involved in the disease process. These observations prompted us to compare these WNV strains with others from different origins. Results are presented and discussed on the ligh of the WNV ecology.

R17.12

NUCLEIC ACID SEQUENCE OF THE VP1 REGION OF ATTENUATED MS-1 HAV.

Betty H. Robertson, Vicki K. Brown, and Daniel W. Bradley. Hepatitis Branch, Division of Viral Diseases, Centers for Disease Control, Atlanta, Georgia 30333, USA.

The nucleotide sequence of the VP1 region of marmoset-attenuated hepatitis A virus (HAV), MS-1, was determined by incorporative dideoxy sequencing of the RNA obtained from purified, liver-derived virus. Comparison of this nucleotide revealed that one of the isolates, HM-175, which was obtained from Australia and passed three times in marmosets, had a 8.5-11% nucleotide variability compared to the remaining four isolates which were isolated from North American sources. This nucleotide variability does not result in significant amino acid differences with the exception of two of the North American isolates, which were derived from tissue culture passage. These isolates have been shown to contain regions of variability generated by nucleotide insertions and/or deletions, while the remaining three isolates, including the Australian isolate, demonstrate limited amino acid differences within the VP1 molecule.

R17.13

ENTERICALLY TRANSMITTED NON-A, NON-B VIRAL HEPATITIS: SERIAL PASSAGE OF DISEASE IN CYNOMOLGUS MONKEYS AND RECOVERY OF DISEASE-ASSOCIATED 27-30nm VIRUS-LIKE PARTICLES.

D. W. Bradley, K. Krawczynski, E. H. Cook, Jr., K. A. McCaustland, and C. D. Humphrey. Hepatitis Branch, Division of Viral Diseases, Centers for Disease Control, Atlanta, Georgia 30333.

An experimental model of enterically-transmitted non-A, non-B hepatitis (ET-NANBH) was established in cynomolgus monkeys. First passage animals were inoculated with two different stool suspensions obtained from human cases of ET-NANBH which originated from Burma and Pakistan. Both inocula contained 27-30nm virus-like particles (VLPs) that were specifically aggregated by acute-phase ET-NANBH sera. Five of the seven monkeys showed elevations of alanine aminotransferase activity between 21 and 59 days after intravenous inoculation. ET-NANBH was subpassaged in 5 of 7 monkeys using pools of stool suspensions from first passage animals. One additional passage of disease in 5 cynos resulted in a significantly shortened incubation period. 27-30nm VLPs similar to those found in the human inocula were observed in stool specimens of first, second, and third passage animals. Liver biopsy specimens obtained from infected cynos revealed intralobular changes that were consistent with a diagnosis of acute hepatitis. Our findings indicate that cynomolgus monkeys are suitable experimental models for studies of human ET-NANBH. The 27-30nm diameter VLPs found in infected human primate stools appear to be etiologically linked to disease.

R17.14

SEROEPIDEMIOLOGICAL STUDY ON HEPATITIS B IN ETHIOPIA

M. Rapicetta*, K. Hailu*, G.B. Rossi*, L. Biasnti*, D. Bakura*, C. Morace*, C. Rosera*, S. Conti*, P. Pasquini*. *Istituto Superiore di Sanità, Rome, Italy. *Military Hospital, Addis Abeba, Ethiopia.

The results obtained from a large scale Hepatitis B seroepidemiological study performed in Ethiopia on 5270 recruits aged 18-30 are reported.

The survey showed overall prevalence values of 10.8% for HBsAg, 61.4% for antiHBc and 73.3% of positivity to at least one marker; which are values of high endemicity. 2.5% of sera resulted positive only to antiHBc and simultaneous presence of antiHBs was demonstrated in 10% of HBsAg positive samples. Geographical and Ethnic variabilities were observed.

522 out of 566 HBsAg positive sera were tested for the presence of HBeAg, antiHBe and HBV-DNA by spot-hybridization assay. The prevalance of HBV-DNA was 19%. This value is similar to that found in asymptomatic carriers of North European countries.

Good correlation was observed between HBV-DNA presence and HBeAg status. 83.8% of HBeAg positive, but only 4.3% of antiHBe positive sera showed the presence of HBV-DNA.

R17.15

COMPLEMENT BINDING TO HEPATITIS A VIRUS CAPSID POLYPEPTIDES ISOLATED FROM IMMUNE COMPLEXES DURING THE COURSE OF INFECTION. Harold S. Margolis, Omana V. Nainan Hepatitis Branch, Division of Viral Diseases, Centers for Disease Control, Atlanta, GA, USA

The mechanisms of hepatocellular injury and viral clearance during acute hepatitis A virus (HAV) infection are unknown. The role of circulating immune complexes (CIC) during the course of HAV infection was examined using a conglutinin based, immunoglobulin-specific immune complex assay. CIC with IgM as the predominant class of antibody were identified during the course of experimental HAV infection in chimpanzees and during naturally occuring disease in humans. Immune complexes were purified from serum obtained during HAV infection by positive affinity chromatography on immobilized conglutinin. Following reduction, proteins were separated by SDS-PAGE, were electrophoretically transferred to nitrocellulose and were identified by immunoblotting. A protein band having a molecular weight of approximately 65 KD was stained by antisera to HAV, HAV capsid synthetic peptides and C3d. No bands corresponding to native VP1, VP2, VP3 (molecular weights 23-33 KD) were initally identified. Subjecting the IC to nucleophilic conditions resulted in the appearance of immunoreactive bands corresponding to VP1, VP2, VP3. These data indicate that during infection, HAV is present in CIC and that complement binds to HAV.

R17.16

NON-A, NON-B VIRAL HEPATITIS IN EGYPT. Ashraf M.K. El-Kady, M. Shaker Ibrahim, and Medhat A. Darwish. Ain Shams University, Faculty of Medicine, and Military Medical Academy, Cairo, Egypt.

A study was carried out on 200 patients of age 20-40 years suffering from acute viral hepatitis. Sera were tested for markers of Hepatitis B (HB_sAg, and IgM - specific anti-HBc) and Hepatitis A (IgM - anti-HAV) by the ELISA technique. Sera negative for the markers of both viruses viz Hepatitis A (HAV) and Hepatitis B (HBV) were subsequently tested for IgM heterophil antibodies against Epstein-Barr (EB) virus by the Monospot slide test to diagnose acute infectious mononucleosis and tested for anti-CMV (IgM) by ELISA technique for the diagnosis of acute Cytomegalovirus (CMV) infection. Non-A, Non-B Hepatitis was diagnosed by exclusion.

The results of the study showed that 133 (66.5%) patients had evidence of HBV infection, while only 9 (4.5%) were diagnosed as HAV infection. EB virus and CMV were the possible etiological agents of acute viral hepatitis in 3.5% and 1% respectively. Accordingly the Non-A, Non-B hepatitis in this study amounts to 24.5% of the acute viral hepatitis. Results will be discussed in the presentation.

R17.17

CHARACTERIZATION OF THE PEPLOMERS OF HUMAN HEPATITIS B VIRUS BY A MONOCLONAL ANTIBODY. M.A. Petit, F. Capel, M.H. Riottot, J. Pillot.
INSERM U 131, Clamart & Microbial Immunology Unit, WHO Collaborating Center for Reference and Research on Viral Hepatitis, Pasteur Institute, Paris, France.

Highly purified hepatitis B virions (HBV) were used as immunogens to produce murine monoclonal antibody secreting hybridomas. One IgG1 monoclonal antibody F222 was shown, by Western immunoblotting analyses, to faintly recognize the monomeric pre$S1$-specific proteins (P39^S and GP42^S), but to react most strongly with two surface antigen glycoproteins with molecular weights 54,000 and 66,000. High avidity of the antibody was also observed with immobilized virus particles in a solid-phase RIA. The reactivity of this monoclonal antibody could be inhibited by native HBV. The binding of F222 was extremely sensitive to trypsin or V8 protease digestion of purified virus.

We have demonstrated that the epitope recognized by this antibody is dependent on the linkage of the pre$S1$-specific glycoprotein (GP42^S) as the HBV attachment protein with another viral protein : (a) the S-specific protein (P24^S) as the main structural envelope polypeptide or (b) a nucleocapsid protein (larger than the major core protein, P22^C) probably exposed on the virus surface. This suggests that a host immune response against these peplomer antigens (54/66-Kd) may neutralise infectious virus.

R17.18

IFN-γ DETECTED IN SERA OBTAINED FROM HBV-CARRIERS.

T. Minagawa, A. Nakane, Y. Maruo. Department of Microbiology, Hokkaido University school of Medicine, Sapporo, Japan.

The level of IFN-γ in sera obtained from healthy persons (n=91) and HBV-carriers (n=45) was investigated by using the radioimmunoassay (RIA) kit for the detection of human IFN-γ (Centocor, PA). Asymptomatic HBV-carriers were selected to be shown carrying only HBV-surface antigen (HBsAg) in their sera and the normal ranges of liver functions. The mean titer of IFN-γ in sera of the controls and HBV-carriers was 0.007 ± 0.020 IU/ml and 0.075 ± 0.035 IU/ml, respectively. The titers of IFN-γ of 71 sera out of 91 sera of the control were less than 0.01 IU/ml and the other 20 sera contained IFN-γ which was very low and appeared transiently. On the other hand, only 2 sera of HBV-carriers were shown to contain IFN-γ less than 0.01 IU/ml. These IFN-γ detected in sera were blocked specifically by monoclonal antibody (B3) against human IFN-γ in the assay of the RIA kit. IFN-γ detected in sera of HBV-carriers might solve the mechanisms of the establishment and the continuance of HBV-carrier state and the pathogenesis of hepatitis by HBV.

R17.19

THE VERO CELL RECEPTOR FOR HEPATITIS B VIRUS SMALL S PROTEIN IS A SIALOGLYCOPROTEIN. K. Komai and M.E. Peeples. Immunology/Microbiology Department, Rush-Presbyterian-St. Luke's Medical Center, Chicago, IL, USA.

Noninfectious, hepatitis B virus surface antigen (HBsAg) particles are able to bind to the Vero, green African monkey kidney-derived cell line but not to 30 other cell lines tested. The HBsAg particles were produced by a CHO cell line transfected with the hepatitis B virus (HBV) small S protein gene, and were purified by affinity chromatography. These HBsAg particles contained only the small S protein and were provided by Genentech, Inc. The biochemical nature of the Vero cell binding site was explored by enzymatic treatment of the cells. Several proteases and neuraminidase destroyed most of the receptor activity. Therefore, the receptor appears to be a sialoglycoprotein. However, it is possible that the proteases removed the sialic acid indirectly by removing the sialic acid-containing glycopeptides from the Vero cell surface. This is not the case, because chymotrypsin treatment of the cell surface destroys receptor activity without significantly affecting the cell surface sialic acid content. Therefore, the Vero cell receptor for the small S protein in HBsAg particles is a sialoglycoprotein.

R17.20

ATTACHMENT OF DNA-CONTAINING HEPATITIS B VIRUS PARTICLES TO A MONKEY KIDNEY CELL LINE. M. J. Bankowski, C.W. Ogaton, P. Pontisso, K. Komai, and M.E. Peeples. Immunology/Microbiology Department, Rush-Presbyterian-St. Luke's Medical Center, Chicago, Illinois, USA.

Infection of established cell lines with hepatitis B virus (HBV) has not been successful, possibly due to the absence of a receptor for the virus. We have found that noninfectious hepatitis B surface antigen (HBsAg) particles will bind to a monkey kidney cell line (Vero) but not to 30 other cell lines tested, including BHK cells. These particles contain only the HBV small S protein and were derived from three sources: recombinant particles from CHO cells (Genentech), recombinant particles from yeast cells (Merck, Sharpe and Dohme; MSD), and purified serum particles treated with pepsin (MSD). Recombinant particles containing the middle S protein (Prof. Tiollais, Pasteur Institut) or the large S protein (MSD) also bound to Vero but not to BHK cells. In addition, Vero cells incubated with serum from chronic carriers bound DNA-containing HBV particles while BHK cells bound significantly less, as detected by an HBV-specific probe on a Southern blot. These results indicate that DNA-containing HBV particles can bind to Vero cells and that sequences unique to the middle S and large S proteins do not interfere with HBsAg or DNA-containing HBV particle binding to Vero cells.

R17.21

REPLICATION OF HBV IN TISSUE CULTURE: IDENTIFICATION OF A NOVEL HBV POLYMERASE. O. Laub and M. Bavand. Department of Genetics, The Weizmann Institute of Science, Rehovot, Israel.

We have developed a human hepatoma cell line which carries a tandemly arranged HBV genome. This cell line constitutively secretes Dane-like particles including all known HBV serological markers.

We have demonstrated, using an activity gel procedure that these HBV - like particles contain a novel polymerase activity capable of synthesizing DNA from either a DNA or an RNA template.

R17.22

Localization with Immunogold Probes of Cytopathic Hepatitis A Virus (HAV) and Viral Antigen in Cell Culture T. Crovaans[1,2], H.A. Fields[2], C.H. Humphrey[2], and M.D. Sobsey[1], [1] University of North Carolina, Chapel Hill, NC, and [2] Centers for Disease Control, Atlanta, GA, USA.

A rapidly replicating isolate of strain HM-175 (PHM-175) was shown to cause a cytopathic effect (CPE) in FRhK-4 cells. Under one-step growth curve conditions, maximum cell-associated infectious virus was found at 24-32 h post infection (PI) and maximum cell-associated viral antigen was detected at 32 h PI. Evidence of CPE was first seen at 36 h PI and by 72 H PI, 20-30% of cells were detached from the monolayer. In order to localize virus and viral antigen, FRhK-4 cells infected with a MOI of 10/PFU cell were prefixed at 36 h PI with paraformaldehyde and low concentrations of saponin (0.05%-0.01%). An indirect labeling technique was employed using monoclonal antibody to HAV followed by 5nm gold-antimouse IgG conjugate. Cells were then fixed by standard electron microscopy techniques and thin sectioned. Within infected cell cytoplasm, numerous antigenic sites were labeled with 6-200 gold particles. Vesicles containing virus particles were observed, although many sites of immunogold staining appear to contain no particles. The localization of HAV particles in cytoplasmic vesicles agrees with other findings for HAV in infected liver tissue and for other picornaviruses in cell culture.

R17.23

SURAMIN INHIBITS ENDOGENOUS DNA-POLYMERASE AND PROTEIN-KINASE OF HUMAN HEPATITIS B VIRUS PARTICLES. R.Fernandez-Muñoz, M.L. Calma. Unidad de Virologia, Hospital "Ramon y Cajal", Instituto Nacional de la Salud, Madrid, Spain.

Suramin, an antitrypanosomal drug, has been reported to have antiRNAdependent-DNApolymerase activity, and recently to inhibit HIV (Human Immunodeficiency Virus) multiplication and the DNA polymerase of Duck Hepatitis Virus.

We have examinated the effect of Suramin on the DNA-polymerase and Protein-kinase of Human Hepatitis Virus (HBV) Dane particles obtained from blood of chronic hepatitis B patiens and purified by sucrose and CsCl gradients centrifugation. We found that Suramin inhibited DNApolymerase at 10 µgs/ml, reaching 50% inhibition at 70-100 µgs/ml, and complete inhibition at 200 µgs/ml. The drug also inhibited the HBV Protein-kinase at 5 µgs/ml, reaching 50% inhibition at 20 µgs/ml.

Conclusions: 1.-Suramin inhibits completely HBV DNA-polymerase at concentrations attained in plasma of patients under treatment with the drug, suggesting it should be tried for treatment of HBV infections. 2. Suramin inhibits HBV associated Protein-kinase as well, indicating that, as in other systems, it interacts with a broad range of proteins.

R17.24

PREVALENCE OF ANTI-DELTA IN VARIOUS HBsAg POSITIVE POPULATIONS IN HUBEI PROVINCE, CHINA. Z.H.Zheng, J....Hu, J.H.Zhang, W.P.Zhu, S.F.Liu. Virus Research Institute, Hubei medical College, Wuhan, China.

Epidemiological studies have shown that hepatitis δ virus exists throughout the world. However, studies on prevalence of anti-δ in China have not extensively been done. In present study, a total of 658 serum samples received from various HBsAg positive populations has been studied on the prevalence of anti-δ in all the parts of Hubei province by ABBOTT anti-delta EIA method. The results are summerized in the table.

	Samples tested	No.of anti-δ positive	%
Asymptomatic HBsag carriers	421	22	5.23
Chronic hepatitis B	157	0	0
Acute hepatitis B	38	0	0
Primary hepatoma	42	4	9.52
Total	658	26	3.95

Among of 26 anti-δ positive patients, 19 of them are male, 7 female. Moreover, most of δ hepatitis patients are in the young ages not over 30 years old. The youngest δ hepatitis patient is a 1½ years old child.

R17.25

LOCALIZATION OF HEPATITIS B VIRUS (HBV) IN A PRIMARY TESTICULAR CANCER. P.Somlo
Department of Laboratories,Division of Microbiology St.Mary's Hospital,Montreal,Québec -Affiliated with Mc Gill University,Montreal,Québec.
An embryonal carcinoma of the testicle developed in a Caucasian 29 years old male,three months after removery from HBsAg positive Hepatitis.Microscopic sections prepared from formalin fixed tumor tissue were stained with anti-HBs antibody and examined by fluorescent microscopy.Appropriate controls including absorption techniques were also performed.The carcinoma cells contained HBsAg. Controls including normal testicles and one other testicular tumor were negative.
The result indicates that the HBV in this case localized in the tumor cells.The role of the tumor will be discussed.
The patient is alive and well,4 years after orchidectomy and chemotherapy.

R17.26

SERO-EPIDEMIOLOGY AND TRANSMISSION OF HEPATITIS A VIRUS AMONG CHILDREN IN THE NEGEV, ISRAEL. B. Sarov, S. Bar-Shany, L. Naggan. Epidemiology Unit, Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer Sheva, Israel.

Blood samples of 5630 children 6 months to 16 years old, representative of various population groups of the Negev Region, were tested for anti HAV (HAVab Abbot). Total prevalence was 41% in both sexes with a strong positive association with age. Wide variations in anti HAV prevalence have been observed in different social environments, with extremely high rates among the Bedouins - 100% at 4 years, compared to an average rate of 11% at ages 1-16 in kibbutzim. Age adjusted rates revealed a strong negative association with socio-economic class as manifested by ethnic origin, crowding index, education level of parents and area of residence. A two year follow-up of sero-negative children 2-18 years old (4453 child/years), showed 3.5% incidence of sero-conversion per year with an inverse association with age. The incidence of clinical episodes of Jaundice observed was 1% and varied with area of residence, with the highest rates in mid to upper social class residence. The sub-clinical to clinical rate was very high at ages 2-5 years (1/32) and much lower at ages 10-18 (1/2.4). These data can serve as a basis for planning preventive strategies both with passive and active immunization.

R17.27

EARLY EVENTS OF HEPATITIS A VIRUS INFECTION IN Frp/3 CELLS
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Department of Public Health, University "TorVergata",Rome,Italy

The early interactions between a fast growing strain of hepatitis A virus (HAV) and Frp/3 cells (Foetal rhesus kidney) have been studied. A first series of experiments has been performed by testing the inhibitory activity on virus attachment to Frp/3 cells of whole membranes extracted and of their protein and lipid components. These fractions were able to bind the virus thus impeding subsequent infection. The attachment of HAV to Frp/3 cells was shown to be inhibited by pretreatment of the cells with proteases, phospholipase A_2 and beta-galactosidase. It can be assumed that the receptor on the Frp/3 cell surface is a complex structure involving many chemical components and the relative participation of these is discussed.

Other investigations have been carried out to study the effect of different lysosomotropic agents on HAV infection. Results suggest that HAV entry in Frp/3 cells follows an intravesicular route since infection is affected in the early stages by lipophilic amines. Further information on HAV entry pathway into Frp/3 cells was obtained by means of different metabolic inhibitors.

R17.28

THE FOLLOWED-UP OBSERVATION OF ANTIVIRAL TREATMENT WITH SMALL DOSAGE OF INTERFERON IN CHRONIC HEPATITIS B(40 CASES).....Wu Zhang-qi; Huang Wun-fang, Xiong Kai-jun, Wang Xin-he, Gin Wei-e. Institute of Virology, Academia Sinica; Tongji Medical University, Wuhan, PR of China.

In this paper, followed-up observation during 6-20 months was taken on 40 cases with chronic hepatitis B, who were treated with small dosage of interferon in a period of 6 months. The result is as follows: 12 of 18 HBeAg positive turned into negative (66.6%), anti-HBe positive appeared in 6 cases, 2 of 3 cases with DNAP positive became negative (66.6%), one case with HBAg positive also became negative, 2 cases with HBV-DNA positive remained unchanged, HBsAg were negative in 17.9%(7/39). Among them, the negative percentage of HBeAg and DNAP were as high as 66.6%, this is a significant result. Again, observation from the antiviral complex effect showed the total effective percentage was as high as 57.5%(23/40). After 14, 15 and 20 months' treatment, 3 cases respectively showed HBsAg, HBeAg, HBcAg, DNAP and HBV-DNA all turned into negative. In contrast, the control group with 12 cases of chronic hepatitis, the negative percentage of HBsAg, HBeAg and DNAP were 0%, 16.6% and 0% respectively.

R17.29

HEPATITIS A VIRUS (HAV) CONCENTRATION BY SHELLFISH.
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Hepatitis A epidemics have been related to shellfish consumption. To provide further evidence of the risk of eating raw mussels, the concentration and persistence of HAV was evaluated in experimentally infected shellfish (Mytilus galloprovincialis).

Marine water and shellfish were obtained from approved areas. Mussels were kept in tanks containing marine water, in which tissue culture, produced HAV had been added to a final concentration of 10^2 TCID50/ml. Water and mussels samples were collected after 1, 4 and 24 hours. At the end of the experiments pseudofeces were collected. Mussels omogenates were prepared using a simplified method for recovery of enteroviruses. Appropriate dilutions of water, mussels and pseudofeces samples were inoculated in tissue culture, using FRp/3 cell line in which HAV gives a cytopathic effect, to test for infectivity.

HAV antigen was undetectable in all samples, when tested with ELISA (Organon). An about 100 fold concentration of HAV infectious particles was found in shellfish collected after 1 hour. No further concentration was detected after 4 and 24 hours. The amount of virus in water decreased, while HAV was detected in higher concentration in pseudofeces.

R17.30

OBSERVATIONS OF HEPATITIS A VIRUS IN CELL CULTURES BY THIN SECTION ELECTRON MICROSCOPE. J. Mao, S. Cuai, X. Yang, and J. S. Mao. Department of Virology, Zhejiang Academy of Medicine, Hangzhou, China.

The location and characters of HAV replication in buffalo green monkey kidney (BGMK) cells were studied with thin section electron microscopic technique.

HAV particles were observed only within membrane bound vesicles in the cytoplasm of the cells but not in the cytoplasmic matrix. The HAV particles were scattered or crowded in these vesicles but not crystalline. Fine fibril-like structures in association with some HAV particles also existed. The HAV containing vesicles were round or oval and could arrange along the nuclear membrane. In these experiments, it was not found that HAV particles could release from the cells. In the cytoplasm of HAV infected cells, there were some enlarged reticuloendothelia which were similar to the HAV containing vesicles both in size and in shape. Within such enlarged reticuloendothelia, HAV particles could even be found. It is possible that the HAV containing vesicles originated from these enlarged reticuloendothelia.

R17.31

STUDIES OF INAPPARENT HEPATITIS A INFECTION. N. Yang, P. Yu, A. Mao, N. Chen, S. Chai, and J. S. Mao. Department of Virology, Zhejiang Academy of Medicine, Hangzhou, China.

Serial sera were collected from persons including patients with hepatitis A and contacts in two waterborne epidemic areas A and B of hepatitis A. Both anti-HAV IgM and anti-HAV antibodies were assayed in sera from healthy persons with symptomless, anicterus and normal SGPT who closely contacted with patients with hepatitis A.

Among 18 healthy persons 12 were anti-HAV IgM positive, 2 were the unsusceptive and 4 were not infected in epidemic A. In epidemic B among 32 healtuy children 3 children were anti-HAV IgM positive, 5 were infected by HAV in past and 24 weren't infected. The incidence of inapparent, subclinical and overt infection was 34.3%, 45.7% and 20.0% of whole infection rate, respectively, in epidemic, and was 25.0%, 50.0% and 25.0%, respectively, in epidemic B. In addition, HAV was isolated from feces of a child with inapparent infection.

The results demonstrated that persons with inapparent infection exist besides patients with overt and subclinical infection during epidemic of hepatitis A.

R17.32

DETECTION OF CIRCULATING HBsAgIgM COMPLEX AND ITS DIAGNOSTIC VALUE IN HEPATTITIS B. L.GEBRESELASSIE. NATIONAL RESEARCH INSTITUTE OF HEALTH. ADDIS ABABA, ETHIOPIA.

In an attempt to study:the diagnostic value of hepatitis B surface antigen IgM complex (HBsAgM) we have tested 120 unclassified HBsAg positive patients, 10 patients who are chronic carrirs of HBsAg and 10 blood donors who are negative for HBsAg using ELISA. In the test we used anti human IgM (anti u) as the solid phase and anti HBs labelled with peroxidase as the conjugate. It was found that 54/120 (45%) of the patients with unclassified hepatitis B are positive for the complex. In 9/10 (90%) of the patients who are chronic carriers of HBsAg the complex was detected and none from the blood donors. The testing of sequential sera samples from the nine patients indicated that the complex could be detected in each patient for atleast a period more than five weeks. The longest time the complex was detected was for eleven months. It is observed that the detection of the complex can serve as an early prognostic marker for the transition of acute hepatitis B to chronic hepatitis B.

R18.1

INCREASE IN SERUM IgG IN MICE CHRONICALLY INFECTED WITH ONE PARTICULAR STRAIN OF SCRAPIE. S.C. Collis, R.H. Kimberlin. AFRC & MRC Neuropathogenesis Unit, West Mains Road, Edinburgh, U.K.

Eight scrapie models in mice were screened for changes in the concentration of IgG in serum. A single radial diffusion assay was used to measure IgG throughout the incubation period which in different models ranged from 125 days to longer than the 600-day observation period. Consistent changes only occurred with the 87V strain of scrapie injected intracerebrally (i.c.) or intraperitoneally (i.p.) into mouse strains of the Sinc genotype p7p7. At the clinical stage of 87V scrapie (270-320 days after i.c. injection), the serum IgG concentration reached 1.5 to 2.0 times the value of age-matched, normal-brain-injected control mice. This increase was not caused by a reduced rate of clearance from blood. The use of monospecific anti-sera to individual IgG isotypes revealed a disproportionately large increase in IgG1 at the clinical stage of scrapie with smaller increases in IgG2b and no change in IgG2a. Large increases in serum IgG concentration also occurred in persistently infected IM mice, 300 - 500 days after i.p. injection of 87V, and these mice did not develop the clinical disease. We conclude that chronic infection with at least some scrapie strains can cause an over production of certain IgG isotypes.

R18.2

MODIFICATIONS TO A SINGLE STRAIN OF MOUSE SCRAPIE ON PASSAGE THROUGH OTHER SPECIES. R.H. Kimberlin, S. Cole and C.A. Walker. AFRC & MRC Neuropathogenesis Unit, West Mains Road, Edinburgh, U.K.

The interspecies transmission of scrapie is frequently associated with exceptionally long incubation periods at first passage in the new host compared to later passages (the species barrier effect). The basis of this was investigated using the 139A strain of scrapie which had been cloned by 3 serial passages in mice at limiting infectious doses. Cloned scrapie was passaged through hamsters (twice) or rats (thrice) and then reisolated in mice. Large species barrier effects were encountered on mouse-to-hamster and hamster-to-mouse passage resulting in the isolation of a mutant strain, 139-H/M, with properties very different from 139A. In contrast, the strain reisolated from rats was indistinguishable from 139A. However, a large species barrier was encountered at the mouse-to-rat passage but not at the rat-to-mouse passage. Hence, the transmission of scrapie between species may be associated with (a) no change in properties or (b) a permanent change in the scrapie genome due to the selection of mutants. A third possibility, the donor species effect, is a temporary change occurring only at first pass in the new host species which is largely or entirely caused by the introduction of material from the previous host.

R18.3

IMMUNOLOGICAL APPROACH TO THE STUDY OF SCRAPIE STRAIN VARIATION AND SAF. R.J. Kascsak, R. Rubenstein, P.A. Merz, R.I. Carp, M. Tonna-DeMasi and R. Fresko. N.Y.S. Institute for Basic Research in Developmental Dis., 1050 Forest Hill Road, Staten Island, N.Y. 10314

Distinct scrapie strains have been identified which can be distinguished by parameters of incubation period, histopathological change and species susceptibility. Specific morphological and biochemical properties have been established for scrapie associated fibrils (SAF) isolated from animals infected with different scrapie strains. Polyclonal and monoclonal antibodies have been raised to PrP, the host coded glycoprotein component of SAF. In this study, these antibodies have been used in various immunoassays to further establish the specific characteristics of SAF associated with the different scrapie strains. A single inbred strain of mouse infected with 3 different scrapie agents was used to examine the influence of scrapie strain. A single scrapie strain passaged in several host species was used to investigate host controls. Specific structural and molecular properties of SAF are strongly influenced by both host and scrapie strain. SAF associated with different scrapie strains are related but distinct structures. The uniqueness of these structures will contribute to our understanding of specific molecular and pathological events occurring in unconventional slow viral diseases.

R18.4

PRODUCTION OF SCRAPIE ASSOCIATED PRP PROTEIN IN BRAIN MACROPHAGE/MICROGLIA CULTURES DERIVED FROM SCRAPIE INFECTED MICE. G.S. Merz, V. Schwenk, T. Denny, H. R. Brown, N. Robakis, P.A. Merz, N. Goller and H.M. Wisniewski. N.Y.S. Institute for Basic Res. in Developmental Dis., 1050 Forest Hill Rd., Staten Island, N.Y. 10314

Brain macrophage/microglia have been isolated from normal and scrapie infected mouse brains. The macrophage/microglia cells are defined as binding Mac-1 antibodies and having the enzyme nucleoside diphosphatase present on the cell surface. The cells have been cultured in vitro, and evaluated for the presence of the scrapie associated proteins by a variety of means. Western blots of whole cell lysates, flow cytometric analysis of immunofluoresence and electron microscopic immunogold labelling have shown that the scrapie protease resistant proteins (PrP) are associated with these cells in cup-like invaginations on the cell surface. In situ hybridization with the scrapie PrP specific DNA probe revealed the presence of specific mRNA in these cells. From these observations we concluded that scrapie cells are actively producing PrP.

R18.5

ATTEMPTS TO DETECT NUCLEIC ACID IN SAF WITH ULTRASTRUCTURE P.A. Merz, R.J. Kascsak, R. Rubenstein, R.I. Carp and H.M. Wisniewski. N.Y.S. Institute for Basic Research in Developmental Dis., 1050 Forest Hill Rd., Staten Island, N.Y. 10314

The nature of the scrapie agent is still an enigma. Separate scrapie strains have different biological properties and neuropathology. Scrapie associated fibrils (SAF) isolated from animals infected with different scrapie strains have differing morphologies, biophysical properties, protease sensitivities, polypeptide numbers, and lectin binding capabilities. Protease resistant proteins are a major component of the SAF and as a host coded protein cannot account for the observed differences. If SAF represent one form of the agent then a nucleic acid within the SAF could account for some of the observed differences. Purified SAF from three scrapie strains with less than 40 ng RNA/ml reveal thin structures associated with the ends of the SAF after treatment with protein denaturants. The thin structures but not the SAF are Zn^{++} and RNase sensitive. Paired helical filaments, an abnormal fibril from Alzheimer's Disease, do not exhibit similar structures. Further studies will elucidate the specificity of this potential nucleic acid intimately associated with SAF.

R18.6

SCRAPIE INFECTED SPLEENS: ANALYSIS OF INFECTIVITY, SCRAPIE ASSOCIATED FIBRILS (SAF), AND SAF PROTEINS (PRP'S). R. Rubenstein, P. Merz, R. Kascsak, C. Scalici, M. Papini and R. Carp, NYS Institute for Basic Research, Staten Island, N.Y., USA.

Spleens from scrapie infected animals contain high amounts of infectivity second only to brain. 139A and ME7 infected mouse spleens contain 2 logs less infectivity (compared to brain) whereas 263K infected hamster spleens contain 5 logs less infectivity. SAF have been detected in the brains and spleens from scrapie infected animals. Spleen SAF detection depends on the route of infection since IP injection of 139A resulted in the initial appearance of SAF 3 weeks before its detection following IC injection. Spleen SAF could not be isolated using protocols suitable for brain SAF. This may reflect differences in the components, quantity, and/or biophysical properties of SAF from spleen vs brain. Using a subcellular fractionation procedure with spleens from ME7 infected mice, PrP has occasionally been detected by Western blot analysis. There appears to be at least 50-100 fold less PrP in spleen compared to brain. The mRNA for this protein, however, has not been conclusively identified in this organ. Our studies further suggest an association between SAF, PrP, and infectivity in spleen. The relationship of infectivity, SAF and PrP is vital to our understanding of the nature of the infectious agent.

R18.7

NEURONAL DEGENERATION: AN INDIRECT EFFECT OF MuLV INFECTION. P.M. Hoffman, E.F. Cimino, J.A. Bilello. VA Medical Center and University of Maryland, Baltimore, USA.

Neurotropic murine leukemia viruses (MuLV) produce a neurodegenerative disease characterized by spongiform vacuolation, gliosis, and neuronal dropout. EM studies demonstrated MuLV replication primarily in endothelial cells, less often in glial cells, and rarely in neurons. The distribution of MuLV proteins in the affected CNS was demonstrated by reaction with a goat anti-Rauscher MuLV p30 or gp70, biotinylated rabbit anti-goat antisera, and an avidin-biotin horseradish peroxidase complex (ABC). MuLV RNA was detected by in situ hybridization using a ^{35}S-dCTP labeled full-length Moloney MuLV cDNA probe. In two different MuLV infections, MuLV p30 and gp70 were first detected in endothelial cells of the cerebellar folia, an area that accumulated considerable MuLV protein and RNA during the course of infection, but never developed vacuolation, gliosis, or neuronal dropout. In contrast, pathological changes were observed 3-4 weeks post-infection in the brain stem, deep cerebellar nuclei, and spinal cord. MuLV p30, gp70, and RNA were found in endothelial cells and some reactive glial cells in these areas but only after the pathology was well established. MuLV p30 was rarely detected in spinal cord and cerebellar neurons; MuLV RNA was detected only in endothelial and glial cells. These data are consistent with an indirect effect of MuLV infection on neurons.

R18.8

PRION PROTEIN GENES AND SCRAPIE INCUBATION TIME. G. A. Carlson, D. Westaway, P. A. Goodman, D. Foster, P. C. Hoppe, S. Prusiner. The Jackson Laboratory, Bar Harbor, Maine, and the University of California, San Francisco.

A gene that is closely linked to, or possibly identical with, the prion protein gene (Prn-p), has a profound effect on scrapie incubation time. Four different allelic patterns of restriction fragment polymorphisms at Prn-p have been detected using PrP cDNA probes. Most inbred strains of mice have the a allele and short (100 to 150 days) scrapie incubation times. I/LnJ mice have an exceptionally long scrapie incubation period (200 - 400 days) and share the b RFLP pattern with P/J and BDP/J. RIIIS/J has the c allele, MA/MyJ and d allele, and these mice have incubation times of 130 and 170 days, respectively. F2 mice produced from NZW/LacJ ($Prn\text{-}p^a$) × I/LnJ ($Prn\text{-}p^b$) were typed for Prn-p and inoculated i.c. with the Chandler isolate of scrapie prions. The times of death for mice of each genotype were: a/a, 152.2 ± 7.9 (n = 24); a/b, 218.6 ± 3.6 (n = 48); and b/b 290.5 ± 9.9 (n = 18). These results demonstrate that the prion incubation time gene is co-dominantly expressed. It is not necessary, however, that the 2 alleles be present in the same cells for the intermediate incubation time phenotype to be expressed. Embryo aggregation chimeras were constructed using C57BL/6J ($Prn\text{-}p^a$, $Gpi\text{-}1^b$) and I/LnJ ($Prn\text{-}p^b$, $Gpi\text{-}1^a$) blastocysts and chimerism assessed using the GPI-1 isoenzyme marker. Scrapie incubation times in six chimeric animals ranged from 177 to 222 days.

R18.9

SCRAPIE AGENT HAS AN INDEPENDENT GENOME. M.E. Bruce, A.G. Dickinson. AFRC & MRC Neuropathogenesis Unit, Edinburgh, UK.

The major evidence that scrapie has its own genome is the existence of many different strains, identified by their incubation periods and pathology in the 3 Sinc genotypes of mouse. By serial passage from a range of scrapie sheep and goats in the 2 homozygotes, C57BL ($Sinc^{s7}$) and VM ($Sinc^{p7}$), about 20 strains have been isolated. Scrapie strain variation is shown not to be specified by the host as follows. 1) Numerous different stable strains have been isolated in each single mouse genotype but the same strain is sometimes isolated in both genotypes. 2) The resolution of a scrapie source into stable strains obeys simple rules, involving selection of strains with shorter incubation periods in the mouse genotype used for passage. Artificial mixed infections can be resolved in the same way. 3) Selection of new mutants has been demonstrated for some strains by changing the Sinc genotype used for passage and for other strains by passaging at higher dose within the same Sinc genotype. These changes are not reversible. 4) Some strains are repeatedly isolated in pairs from different natural cases, suggesting a relationship at the molecular level. 5) The same set of strains have been isolated after very different passage histories in experimental sheep and goat lines, showing that mixtures can be copassaged many times and still retain their identity.

R18.10

LINKAGE OF THE SAF PROTEIN (PrP) GENE AND Sinc USING CONGENIC MICE AND RFLP ANALYSIS. N. Hunter, J. Hope, & A. G. Dickinson. AFRC & MRC Neuropathogenesis Unit, West Maine Road, Edinburgh. U.K.

Sinc, with two alleles p7 and s7, is the major gene determining the incubation period of all strains of scrapie in mice. The major protein (PrP) of scrapie-associated fibrils (SAF) is encoded by a cellular gene and we have used a cDNA copy of the hamster PrP mRNA to do RFLP analysis of different inbred mouse strains including VM ($Sinc^{p7}$) and VM ($Sinc^{s7}$) congenic mice. The VM ($Sinc^{s7}$) congenic mice were produced by 19 generations of backcrossing to VM mice the progeny of an original VM x C57BL ($Sinc^{s7}$) cross.

In VM mice, a 5.5kb XbaI fragment hybridises to the PrP cDNA sequence whereas VM ($Sinc^{s7}$) congenic mice have a 3.8kb XbaI fragment. The VM x VM ($Sinc^{s7}$) congenic F1 mice have both the 5.5kb and the 3.8kb fragments. The $Sinc^{s7}$ donor mouse strain, C57BL, has the 3.8kb fragment hence the Sinc gene and the PrP gene are linked, and may even be the same gene. Other $Sinc^{s7}$ inbred mice (IM and MB) have the 5.5kb fragment but so too do some $Sinc^{p7}$ strains (RIII and VL) indicating that the XbaI site polymorphism is not functionally involved in the difference between the two Sinc alleles. We have mapped the polymorphic XbaI site to the 3' flanking region of the PrP gene.

R18.11

EXPRESSION AND STRUCTURAL SIMILARITIES BETWEEN TWO PROTEINS FORMING AMYLOID FIBERS IN THE BRAIN N.K. Robakis, NYS Office of Mental Retardation and Dev. Disab., Inst. for Basic Research, Staten Island, NY, USA.

PrP proteins form amyloid fibers in the brains of several mammalian species affected by unconventional slow viruses. The cerebrovascular and plaque amyloid peptide is accumulated in the brains of people affected by Alzheimer disease (AD) and Down syndrome. Although the etiologies of these diseases seem to be different, there are several similarities in the expression pattern and in the structure of these two proteins. Evidence indicates that 1) the levels of the mRNAs encoding these proteins are unaffectd by the disease; 2) these mRNAs are not specific to the brain and are synthesized by many different cell types; 3) the genes encoding these proteins are conserved among mammalian species; 4) both proteins are largely hydrophilic with a highly hydrophobic region at or near the carboxy-terminus; 5) the peptides forming the amyloid fibers are cleaved from their respective precursors post-translationally and 6) the amino acid sequences forming the amyloid peptides are followed by trans-membrane regions. In humans, however, the genes encoding these proteins are located on two different chromosomes and there is no significant homology between their primary structure. It is possible that similar post-translational modifications trigger the formation of amyloid fibers by these two proteins.

R18.12

EVIDENCE FOR GLYCOLIPID MODIFICATION OF THE SCRAPIE PRION PROTEIN. Neil Stahl, Bradford W. Gibson, and Stanley B. Prusiner. University of California, San Francisco, CA, USA. 94143-0518

We are using HPLC, GC/MS, and LSIMS to identify post-translational modifications of purified PrP 27-30 which may play a role in scrapie pathogenesis and explain the differences in physical properties between PrP^C and PrP^{Sc}. During purification, PrP^{Sc} is converted to PrP 27-30 by limited proteolysis. We have detected ethanolamine, P_i, inositol, and stearic acid as the trimethylsilyl derivatives by GC/MS following acid hydrolysis of PrP 27-30 in 6 N HCL at 110 °C for 24 hours. These compounds persist despite boiling in SDS, repeated chloroform/methanol extractions, or gel purification of PrP 27-30. These components are diagnostic for the presence of a glycolipid linked to the carboxy terminus of a variety of proteins including Thy-1, acetylcholinesterase, and Trypanosome VSG. These proteins as well as PrP have short, hydrophobic carboxy-terminal segments. Denatured PrP 27-30, as well as PrP^C, is resistant to carboxypeptidase, supporting the existence of a blocked C-terminus. Although PrP 27-30 contains N-linked oligosaccharides, there is no evidence for O-linked glycosylation or phosphorylated amino acids because limited acid hydrolysis of PrP 27-30 (6 N HCl at 110 °C for 2.5 hours) failed to generate peaks corresponding to galactosamine or phosphorylated serine, threonine, or tyrosine upon HPLC analysis of the PITC derivatives.

R18.13

EXPRESSION OF PRION PROTEIN (PrP) IN PRIMARY CULTURES OF NEONATAL HAMSTER BRAIN. D.R. Borchelt, M.P. McKinley, W.C. Mobley and S.B. Prusiner. University of California, San Francisco, CA, USA. 94143-0518

Scrapie infectivity is inseparable from a modified product of a normal and developmentally regulated host gene designated PrP. In hamster brain, detectable levels of PrP mRNA are present 1 day after birth and rise to adult levels by 20 days. If neonatal hamsters are given nerve growth factor (NGF) injections, increased levels of PrP mRNA are detected in developing hamster forebrain. We investigated expression of PrP mRNA and protein in cultured cells derived from one day old hamster brains. PrP mRNA and protein increase rapidly in cultured cells after plating and reach adult brain levels within 9 days. Culture conditions which favored the growth of differentiated (non-replicating) neurons resulted in the greatest expression of PrP mRNA. Passage of primary cultures resulted in a greater than 10-fold reduction in the level of PrP mRNA. In primary brain cultures treated with 100ng/ml NGF, an increase in PrP mRNA and protein levels during the first 7 to 9 days of cultivation was observed. Consistent with a neuronal site of action, NGF treatment did not alter PrP expression in three transformed hamsterglial cell lines. These data suggest that the highest level of PrP expression occurs in neurons and that NGF can modulate PrP synthesis in cultured cells derived from neonatal hamster brain.

R18.14

ATTEMPTS TO DISINFECT SURFACES CONTAMINATED WITH ETIOLOGICAL AGENTS OF THE SPONGIFORM ENCEPHALOPATHIES. D.M. Asher, K.L. Pomeroy, L. Murphy, C.J. Gibbs, Jr., and D.C. Gajdusek. National Institutes of Health, Bethesda, Maryland, USA.

The number of reported iatrogenic cases of Creutzfeldt-Jakob disease (CJD) continues to increase. The etiological agents of that disease and of the other spongiform encephalopathies are extremely resistant to inactivation by chemical disinfectants and physical methods of sterilization used in hospital and laboratory practice. Previous studies on inactivation of the agents of scrapie and CJD suspended in diluent suggested that 1 hr exposure to solutions of either sodium hydroxide (1N or greater) or sodium hypochlorite (2.5% or greater), or to steam at a temperature of 132 C were adequate to inactivate large amounts if not all detectable infectivity. We examined the effects of those and other regimens on the infectious agents under conditions more closely simulating those of actual use—dried onto surfaces. Measured amounts of brain suspensions from rodents infected with the agents of scrapie and CJD were dried onto glass and steel objects. The amount of infectivity adhering to the surfaces was assayed, and replicate objects were exposed to various regimens of heat sterilization or disinfectants and then examined for residual infectivity. Although treatments with hydroxide, hypochlorite and 132 C steam reduced the number of contaminated objects, no regimen was completely effective in decontaminating all replicates, not even exposure to steam for 2 hr. Other regimens were completely unsuccessful. The resistance of at least some fraction to inactivation by heat and chemicals may be even greater when agents of the spongiform encephalopathies are dried onto surfaces than when they are suspended in liquid. It is imperative that fully reliable methods of decontamination be devised.

R18.15

EXPRESSION OF THE HAMSTER PrP GENE USING EUKARYOTIC EXPRESSION VECTORS. M. Scott, D. Borchelt, D. Bredesen, D. Butler, K. Hsaio and S.B. Prusiner University of California, San Francisco, CA, USA. 94143-0518

Screening of several mammalian cell lines has identified the mRNAs encoding the hamster and mouse prion proteins (PrP) at levels similar to those found in the brains of adult rodents, but PrP is undetectable in most cases. Introduction of the hamster PrP gene fused to several different eukaryotic promoters similarly failed to yield detectable PrP. Expression of the hamster PrP mRNA at levels over 100-fold higher than those detected in adult brains using a *Spodoptera* cell baculovirus vector system yielded PrP at levels similar to those found in hamster brains. The low level of PrP produced in relation to the level of mRNA compared to hamster brains is not a consequence of protein instability, since pulse chase experiments showed that the PrP expressed in baculovirus infected cells was extremely stable. We conclude that PrP mRNA is translated inefficiently. Immunofluorescence studies revealed that PrP was expressed on the surface of the recombinant baculovirus infected cells but was absent from control cells. Immunofluorescence was sufficiently sensitive to identify PrP at varying levels on the surfaces of several mammalian cell lines even where PrP protein was undetectable by Western blot. Efforts to remove the block in translation and to achieve efficient expression of hamster PrP are in progress.

R18.16

NUCLEOTIDE SEQUENCE ANALYSIS OF *Prn-p* GENES FROM SHORT AND LONG SCRAPIE PRION INCUBATION PERIOD MICE. D. Westaway, P. Goodman, C. Mirenda, G. Carlson, and S.B. Prusiner. University of California, San Francisco, CA, USA 94143-0518 and Jackson Laboratory, Bar Harbor, ME, USA 04609

Biochemical evidence implicates a protein, PrP^{Sc} as the major and possibly sole component of the scrapie prion. PrP^{Sc} is related to a benign cellular protein, PrP^{C}; both PrP isoforms are encoded by the same cellular murine gene, *Prn-p*. Mendelian analysis of prototypic short (NZW:113 days), and long incubation period mice (I/LnJ:200-385 days) has defined a single autosomal dominant gene, *Prn-i*, as the major determinant of scrapie incubation period lengths. A *Prn-p* Xba I restriction fragment length polymorphism (RFLP) cosegregates with the incubation period phenotype, establishing linkage between *Prn-p* and *Prn-i*. (Carlson *et al.*, Cell 46,503, 1986). We have isolated molecular clones of chromosomal *Prn-p* genes from NZW and I/LnJ mice, and have defined a restriction/modification phenomenon which has interfered with previous attempts to clone PrP genes propagated in E. coli. Restriction mapping of these clones reiterates that the gross structure of *Prn-p* genes is the same in NZW and I/LnJ mice (Westaway and Prusiner, NAR 14,2035, 1986). Coding sequences of these *Prn-p* alleles will be presented, and the possible congruence of *Prn-p* and *Prn-i* discussed with respect to this data.

R18.17

PROPERTIES OF SCRAPIE PRION PROTEINS INTO DETERGENT-LIPID-PROTEIN COMPLEXES. R. Gabizon, M.P. McKinley, D. Groth, L. Kenaga and S.B. Prusiner. University of California, San Francisco, CA, USA. 94143-0518

The scrapie prion protein (PrP^{Sc}) as well as a smaller protein (PrP 27-30) derived by limited proteolysis, are membrane proteins which aggregate into rods upon detergent extraction. Upon mixing purified scrapie amyloid rods with phosphatidylcholine (PC) and cholate, detergent-lipid-protein (D-L-P) complexes were formed. Removal of the detergent by dialysis created liposomes. Despite vastly different morphologies, the rods, D-L-P complexes and liposomes all exhibited scrapie infectivity. Mixing PC with other lipids, phosphatidylglycerol (PG), or phosphatidyl-serine (PS) did not alter the scrapie prion titer; however, lipid mixtures containing phosphatidyl ethanolamine (PE) did exhibit lower titers. Mixtures of PC and dolicol or dolicol-phosphate (the latter known to increase membrane fluidity) failed to alter scrapie prion infectivity of D-L-P complexes. PrP 27-30 incorporation into liposomes was found to be reversible; the rods were reformed and scrapie infectivity preserved after chloroform-methanol extraction of liposomes. PrP^{Sc} was also incorporated in liposomes by the procedure used for PrP 27-30. Moreover, phospholipids mixed with microsomes containing PrP^{Sc} prevented aggregation of PrP into rods upon extraction with detergents.

R18.18

DEVELOPMENTAL REGULATION OF SCRAPIE INCUBATION TIMES IN NEONATAL GOLDEN HAMSTERS M.P. McKinley, W.C. Mobley, S. Coleman, M. Peterson and S.B. Prusiner. University of California, San Francisco, CA, USA 94143-0518

Scrapie infectivity is inseparable from PrP^{Sc} an abnormal isoform of the cellular prion protein PrP^{C}. Earlier studies demonstrated hamster brain PrP mRNA is first detectable at very low levels in 1 day old animals and then it increases to a maximal level by 20 days of age. During development, PrP mRNA expression is differentially regulated in various brain regions. Notably, PrP mRNA levels in basal forebrain neurons were significantly increased by exposure to nerve growth factor (NGF). Since Hotchin and Buckley (Science 196:668, 1977) reported that the onset of clinical scrapie in mice could be delayed by inoculation of prions IP into animals less than 18 h old, we investigated the possibility that low levels of PrP^{C} might be responsible for the prolongation of scrapie incubation times. Unexpectedly, we found that the onset of clinical signs in hamsters was reduced from 77±2 to 63±2 days if IC inoculations into neonatal animals were performed before 7 days of age. IP inoculations into neonates reduced onset of scrapie from 125±5 to 87±2 days. Intervals from inoculation to death were reduced concomitantly. Acceleration of scrapie in newborn animals was abolished when intraventricular injections of NGF were given 2 days prior to inoculation. These observations suggest that the level of PrP^{C} expression may modulate the length of the scrapie incubation period.

R18.19

Scrapie, a virus-induced amyloidosis of the central nervous system.
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The role of scrapie associated fibrils (SAF) in the pathogenesis of spongiform encephalopaties caused by unconventional viruses has been studied for scrapie in hamsters and mice. Kinetic data on the development of infectivity, SAF, and disease in brain as well as quantitative studies on SAF and infectivity in brains and spleens clearly demonstrate that SAF and the disease inducing virus are separate entities. The results furthermore show that SAF-formation succeeds virus formation but preceds clinical disease.

R18.20

DIVERSITY OF CHARACTERISTICS OF SCRAPIE ISOLATES FROM FIVE SUFFOLK SHEEP. R.I. Carp, S.M. Callahan, Y.S. Kim and R.C. Moretz, New York State Institute for Basic Research in Developmental Disabilities, Staten Island, New York, USA.

Brain homogenates from five Suffolk sheep with clinical scrapie were passaged in an s7s7 (C57BL) and a p7p7 mouse strain (VM or IM). The five passage lines in C57BL mice (one from each sheep) yielded shorter incubation periods in s7s7 strains than in p7p7 strains. Two of the five lines that were passaged in a p7p7 strain had shorter incubation periods in p7p7 than in s7s7 strains. The other three p7p7-passaged lines had shorter incubation periods in s7s7 strains. Most of the isolates produced amyloid plaques in the brain, at least through the third passage in mice. Two of the isolates passaged in C57BL mice produced marked increases in weight during the preclinical phase of disease. The average weight of these mice was almost double that of controls. Many of the obese mice had aberrant glucose tolerance test results, with approximately 20% classified as diabetic (defined as 5 S.D. above normal). The differences in the characteristics of these passage lines can be attributed to: (1) differences in the source of the scrapie isolates, and (2) passage of the isolates in different mouse strains.

R18.21

THE SCRAPIE MEMBRANE HYPOTHESIS TWENTY YEARS ON.
G. D. Hunter, 21 Cleveland Grove, Newbury, Berks, England.

The scrapie prion protein crosses the cell membrane twice and fulfils some of the predictions of the scrapie membrane hypothesis (Gibbons & Hunter, Nature, 1967, 215, 1041). But it appears to have the same amino-acid sequence as the corresponding normal brain protein. Unless a constraining nucleic acid component can be found, the altered properties of the scrapie prion protein are best explained by the membrane hypothesis, i.e. as arising from altered oligosaccharide patterns on the surface of the prion protein. Various detailed possibilities and their practical consequences will be discussed.

R18.22

THE HYPOTHALAMUS AND ADRENAL GLANDS PLAY A ROLE IN SCRAPIE INDUCED OBESITY. Y.S. Kim, R.I. Carp, S.M. Callahan, and H.M. Wisniewski. NYS Institute for Basic Research in Developmental Disabilities, Staten Island, New York, USA.

In previous studies we found an increase in total body weight during the preclinical phase of disease in certain scrapie strain-mouse strain combinations. This work was done with routine intracerebral injection.

In the present study, ME7 scrapie agent (5 ul of a 1% brain homogenate) was injected stereotaxically in the hypothalamus or cerebral cortex of SJL and C57BL mice. In the SJL-ME7 combination there was a higher degree of obesity in the hypothalamic injection group than in the cortex injection group. Weight of both groups were significantly greater than controls injected with normal mouse brain. For the C57BL-ME7 combination, obesity developed only in the hypothalamus injection group. Adrenalectomized SJL and C57BL mice were injected with ME7 in the hypothalamus. Sham operated controls showed a significant increase in body weight, whereas adrenalectomy prevented the weight increase. Our results suggest that scrapie induced obesity depends on the hypothalamic-pituitary-adrenal axis and that this may be a useful experimental model to study obesity pathogenesis.

R18.23

AMPHOTERICIN B DELAYS THE REPLICATION OF SCRAPIE AGENT IN BRAIN OF INFECTED HAMSTERS. M Pocchiari, S Schmittinger, A Ladogana, P Casaccia, C Masullo. Institute of Neurology, Catholic University, Rome, Italy.

Experimental scrapie in hamster is considered an excellent model for the study of the unconventional slow viral infections naturally occurring in animals and man (Creutzfeldt-Jakob disease). Recently we found that amphotericin B (AmB), a polyene antibiotic, is able to increase the incubation time in hamsters receiving the scrapie inoculum by intracerebral route (Pocchiari et al., 1987). Under AmB treatment (1 mg/Kg/6 days a week) the incubation time increased proportionally with the length of the treatment. The effect of AmB is dose-dependent. The four doses tested (0.1, 0.5, 1 and 10 mg/Kg) were all effective, but with increased potency. AmB treatment is more effective during the first week after inoculation than during the second or the fourth week and is ineffect during the fifth week after inoculation or when started in the two weeks before scrapie injection. AmB decreases the rate of replication of the scrapie agent in hamster brain althought it does not reduce the final titer of the agent. Our data suggest that AmB interacts with the scrapie agent on the plasma membranes of the CNS target cells, interfering with the agent replication. However we do not think that this drug could be of any help in the treatment of Creutzfeldt-Jakob.

R18.24

DETECTION OF SAF PROTEIN IN MOUSE ORGANS AFTER SCRAPIE INFECTION. M. Shinagawa, S. Doi, M. Ito, H. Goto, G. Sato. Department of Veterinary Public Health, and Department of Veterinary Microbiology, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Japan.

A simple method to prepare fractions for the detection of SAF protein from small amounts of scrapie mouse tissues was developed. Recovery rate of SAF protein from brain was more than 63%, but less from spleen (55%) and lymphnodes(40%) without using ultracentrifugation.

Groups of 9 mice each infected intraperitoneally with Obihiro strain of scrapie agent were sacrificed at intervals of 6 weeks and detection of SAF protein in brain, spleen, lymphnodes, and white blood cells was carried out using the antiserum. In the brain, SAF protein was detected starting from 24 weeks following infection, when the onset of the clinical symptoms was observed in some mice. From 6 weeks after infection, the protein was detected in the spleen, and concentration of the protein in the organ seems to remain roughly constant during the course of the infection. It was difficult to obtain enough lymphnode materials, however, the protein was detected in the samples prepared at 6, 18, and 24 weeks after infection. We failed to detect SAF protein in the white blood cells at any stage of the infection.

R19.1

RELATIONSHIPS AND FUNCTIONS OF VIRUS DOUBLE-STRANDED RNA IN A KILLER STRAIN OF USTILAGO MAYDIS. S.L. Shelbourn, P.R. Day[1], K.W. Buck. Department of Pure and Applied Biology, Imperial College of Science and Technology, London SW7 2BB, U.K. and [1]Plant Breeding Institute, Cambridge CB2 2LQ, U.K.

DsRNA from virus particles isolated from a P4 killer strain (77) of Ustilago maydis, which secretes a protein toxin of apparent mol. wt 10K, was resolved by PAGE into 8 segments with sizes (kbp) 6.7 (H1), 4.5 (H2), 3.2 (H3a), 3.1 (H3b), 2.6 (H4), 0.98 (M2-4), 0.92 (M3-4) and 0.36 (L1). A derivative isolate (77-3) had lost the H2 and M3-4 segments but still produced virus particles and protein toxin. Northern hybridization showed that dsRNA segments M2-4 and L1 are closely related but no homology was detected between H1, H3a, H3b and H4, or between any of these H segments and M2-4 or L1. When individual denatured dsRNA segments were translated in vitro in a reticulocyte lysate system H1, H3a, H3b and H4 each give rise to polypeptides with mol. wts > 93K. Evidence has been obtained that a mixture of viruses, or virus strains, is present in isolate 77 and it is proposed that each H segment encodes a distinct virus capsid polypeptide. In vitro translation of denatured M2-4 gave rise to a polypeptide of apparent mol. wt. 13K, which was immunoprecipitated by antiserum to the secreted protein toxin and is therefore probably a toxin precursor. No in vitro translation product was detected from denatured L1.

R19.2

HYBRID-STIMULATED TRANSLATION OF THE IN VITRO TRANSCRIPT OF M dsRNA OF THE KILLER VIRUS OF YEAST. Iffat Hussain and Michael J. Leibowitz. Dept. of Molecular Genetics and Microbiology, UMDNJ-Robert W.J. Medical School, 675 Hoes Lane, Piscataway, New Jersey 08854, U.S.A.

The m transcript of the M dsRNA of killer virus of the yeast, Saccharomyces cerevisiae can be translated in a yeast cell-free system (1) to yield the polypeptide precursor to toxin and resistance substance. The 5'-terminal region of m transcript comprises a hairpin structure and contains sequences near the translation initiation site that are complementary to sequences near the 3'-termini of yeast 18S and 5.8S rRNAs. Synthetic olideoxynucleotides of various lengths and complementary to the proposed rRNA binding site hybridized to m and inhibited its translation in vitro. Olideoxynucleotides with different sequences hybridizing to the distal side of the hairpin, not involving the initiating AUG, stimulated translation at critical molar ratios of olideoxynucleotide to transcript.

In the presence of the elongation inhibitor, anisomycin, ribosomes bound to m to form stable initiation complexes. These complexes will be used to study message-ribosome interactions.

(1) Iffat Hussain and Michael J. Leibowitz. Gene 46 (1986), 13-23.

Supported by USPHS Grant GM32413.

R19.3

SEQUENCE RELATIONSHIPS BETWEEN DOUBLE-STRANDED RNA SEGMENTS FOUND IN NATURALLY OCCURRING ISOLATES OF Rhizoctonia solani. N. Bharathan B. P. Bandy, S. M. Tavantzis. Department of Botany and Plant Pathology, University of Maine, Orono, Maine, USA.

Double-stranded (dsRNA) from field isolates of R. solani belonging to different Anastomosis Groups (AG's) and of varying degree of virulence were fractionated by agarose gel electrophoresis, transferred electrophoretically to Hybond™ nylon transfer membrane, and hybridized under low or high stringency conditions to various probes. Probes were individual dsRNA segments selected on the basis of size as well as Anastomosis groupings and degree of virulence of the fungal isolates from which they were derived. The ds-segments used as probes were eluted from low-melting temperature agarose gels, alkali hydrolyzed, and 5' end labeled with ^{32}P by T_4-Polynucleotide Kinase. Preliminary data suggests that the majority of ds-RNA segments from the isolates of the same AG have considerable base sequence homology. Experiments are presently carried out dealing with the use of ds-RNA probes originating from isolates of AG's other than that to which the fractionated and blotted ds-RNAs belong.

R19.4

WITHDRAWN

R19.5

CHARACTERIZATION OF DOUBLE-STRANDED RNA GENETIC ELEMENTS ASSOCIATED WITH BIOLOGICAL CONTROL OF *ENDOTHIA PARASITICA*. D. L. Nuss. Roche Institute of Molecular Biology, Roche Research Center, Nutley, New Jersey 07110 U.S.A.

Double-stranded RNA genetic elements associated with a North American hypovirulent strain (Grand Haven 2 (GH2)) of *Endothia parasitica* have been analyzed at the RNA level. A number of structural landmarks, including 3'-polyadenylic acid base paired to 5'-polyuridylic acid, were identified. cDNA libraries of GH2 dsRNAs and the dsRNAs associated with a European hypovirulent strain, EP713, have been constructed. These cDNA libraries are being characterized in an effort to identify and determine the mechanism of expression of hypovirulence associated dsRNA specified gene products.

R19.6

TRANSCRIPTION AND TRANSLATION OF USTILAGO MAYDIS VIRUS. R. F. Bozarth, C. K. Podila, S. W. Yie, and W. H. Flurkey. Departments of Life Sciences and Chemistry, Indiana State University, Terre Haute, IN 47809, U.S.A.

In vitro transcription of the double-stranded RNA mycovirus of Ustilago maydis resulted in daRNA and saRNA. The daRNAs were determined by characteristic mobilities in gel electrophoresis, lack of sensitivity to ribonuclease and specific hybridization tests. The saRNAs were identified by elution from a CF-11 column and by their sensitivity to RNAse. Partial length transcripts were produced from H daRNA segments and full length transcripts were produced from M and L segments. Since only the positive strand of M2 daRNA was labeled by the in vitro reactions and daRNA labeled in vivo with ^{32}P UTP could be chased from the daRNA with unlabeled UTP, it was concluded that transcription occurred by strand displacement.

In vitro tranalation of the plus strand resulting from in vitro transcription of M2 segment of daRNA yielded an approximately 24 kD protein.

In vitro tranalation of purified daRNA segments yielded capsid protein when the H2 segments of strains P1 and P4 or the H1 segment of strain P6 were used. All in vitro-produced capsid proteins reacted with native capsid protein antisera prepared against all of the three strains.

R19.7

HOMOLOGY BETWEEN AND TRANSMISSION OF DSRNAS FOUND IN BOTRYODIPLODIA THEOBROMAE. D. M. Mathews and J. A. Dodds, Dept. Plant Pathology, University of California, Riverside, CA 92521, USA.

DsRNAs purified from 39 isolates of Botryodiplodia theobromae were resolved by polyacrylamide gel electrophoresis into several groups based on their number (between 1 and 14) and size (MWt between 4.0 and 0.2 x 10^6). Four different dsRNAs [Wt of 4.0 x 10^6 (2 examples), 1.0 x 10^6 (one example consisting of several similarly sized dsRNAs), and 0.5 x 10^6] were purified and used to make randomly primed cDNA. When used in northern analyses against dsRNAs from all 39 isolates, some homologies were detected, but it was also concluded that dsRNAs of similar size did not necessarily share sequence homology.

The dsRNAs are readily transmitted through conidia. Single spore subcultures were obtained from 11 parental isolates, each of which had different patterns of dsRNAs. The transmission of the parental dsRNA patterns to the subcultures was 100% for 10 of the isolates. Eighty five percent of the subcultures derived from the 11th isolate had fewer dsRNAs than the parent.

Inoculation assays on yams and apples showed no consistent evidence for a correlation between the presence of dsRNA and virulence in these hosts.

R19.8

TRANSCRIPTION OF DOUBLE-STRANDED RNA IN VIRIONS OF HELMINTHOSPORIUM VICTORIAE 190S VIRUS. S. A. Ghabrial, Department of Plant Pathology, University of Kentucky, Lexington, USA.

The virion-associated RNA polymerase activity of the 190S virus from H. victoriae was shown to catalyze in vitro synthesis and release of full-length ssRNA transcripts of genomic dsRNA (MW 3.0 x 10^6). The transcriptase activity required all four nucleoside triphosphates and magnesium ions. Electrophoretic analyses of radiolabelled RNA polymerase products, performed in 4% polyacrylamide gels containing 8 M urea, revealed a major ssRNA product of MW 1.5 x 10^6 which comigrated with denatured 190S-dsRNA. All labelled RNA products were shown to be single stranded, since no label was detected by autoradiography when the reaction products were first incubated with RNase in either high or low salt, then subjected to electrophoresis. Evidence that the in vitro ssRNA transcripts were released from the particles was based on electron microscopy of transcribing particles and on time course analysis of total reaction mixture by agarose gel electrophoresis. Thus, transcription of the 190S dsRNA appears to occur conservatively because, in the presence of [^{32}P-α] UTP, parental duplex remained unlabelled and the labelled full-length ssRNA transcripts were released from the part[illegible]

R19.9

A MYCOVIRAL DOUBLE-STRANDED RNA GENOME

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The fungal plant pathogen Periconia circinata is the host to several unrelated viral systems, with each viral system consisting of one or more dsRNA species. A cDNA library to a viral system consisting of a 2.0 kb and 1.8 kb dsRNA has been constructed and is being sequenced. The informational context of the genome and its organization will be discussed. The viral 2.0 kb dsRNA is not simply a pliable linear helix. Structural properties of the 2.0 kb dsRNA include sequence-directed bending and polymorphism.

R19.10

STRUCTURE AND FUNCTION OF A VIRUS ENCODED MYCOTOXIN. Y. Koltin, I. Ginsberg, T. Peery, R. Steinlauf, N.I. Smorodinsky. Department of Microbiology, Faculty of Life Sciences, Tel Aviv University, Ramat Aviv, Israel.

The viruses of the fungus Ustilago maydis encode toxins that are effective against sensitive strains of the same species and related species. Of the 3 toxin types known, each expressed by a different virus subtype, the toxin encoded by the subtype P6 is the best characterized. The toxin consists of two small polypeptides (10 and 12.5 Kd) known as VP10 and VP12.5. The polypeptides are not covalently linked and affect the target cell sequentially in a defined manner. The effect of the toxin on spheroplasts indicates that the effective interaction with the target cell requires some earlier interaction on the cell wall.

Monoclonal antibodies derived against VP12.5 are currently used for affinity purification of the two components and for the identification of the precursors of each component. Two of the monoclonal antibodies appear to be different and are used in studies of the biologically active epitopes of VP12.5.

R19.11

M dsRNA INDUCES MAK16 TRANSCRIPTION: VIRAL INDUCTION OF A HOST GENE NEEDED FOR VIRAL REPLICATION. R. B. Wickner, National Institutes of Health, Bethesda, Maryland, U.S.A.

There are five families of dsRNA that replicate in S. cerevisiae, three of which (L-A, L-BC, and M) are found as intracellular virus-like particles (VLPs). The replication of L-A, L-BC, and M dsRNA occurs within these VLPs. The replication of L-A, L-BC, and M dsRNA is repressed by the chromosomal SKI gene products and depends on the products of various chromosomal genes (MAK, MKT, and CLO genes).

The MAK16 gene (among others) is necessary for M dsRNA replication as well as for cell growth. The MAK16 gene has a 306 amino acid open reading frame with a highly acid region (25 acidic residues in a 41-residue stretch) and two tandem potential tyrosine-phosphorylation sites. The MAK16 message is induced about 5-fold by the presence of M dsRNA. 10-fold derepression of M dsRNA replication that results from a ski$^-$ mutation does not further induce MAK16 transcription.

Cloning of SKI8 (Sommer and Wickner, in press) and SKI3 has shown that these genes are nonessential except for their essential role in protecting the host from the cytopathology produced by derepressed M dsRNA replication.

Point mutants in the mkt1 gene are temperature-sensitive (ts) for their maintenance of M_2 dsRNA if L-A-HN is present. Deletion mutants constructed using a cloned MKT1 gene show the same ts phenotype for M_2 replication.

R19.12

MOLECULAR CLONING AND DETECTION OF AGARICUS BISPORUS DOUBLE-STRANDED RNA. M.C. Harmsen and J.G.H. Wessels. Department of Plant Physiology, University of Groningen, Kerklaan 30, Haren, The Netherlands.

Severely diseased 'virus-infected' sporophores of the cultivated mushroom (*Agaricus bisporus*) contain up to 10 major dsRNA segments (of 0.80, 0.84, 1.26, 1.55, 2.26, 2.30, 2.50, 2.60, 2.80 and 3.5 kb). Healthy mushrooms without noticeable symptoms at least contained one of these dsRNAs (L6 dsRNA, 2.26kb). The dsRNAs showed no homology by hybridizations. Most of the dsRNAs encode proteins as shown by *in vitro* translations. These yielded at least 7 discrete polypeptides, ranging from 20,000 to 130,000 mol.wt. This covers approximately 70% of the coding capacity of the major dsRNAs. For development of a diagnostic test a cDNA library of the dsRNAs was made. Size-fractionation of double-stranded cDNA on LMP agarosegels prior to dC-tailing and annealing in dG-tailed pUC9 was neccesary to obtain full-sized clones. Down to 1 pg of dsRNA sequence could be detected when using nick-translated cDNA clones as hybridization probes. The cDNA clones, with the exception of L6 dsRNA clones, hybridize specifically with nucleic acid preparations isolated from diseased mushrooms or mycelia.

R20.1

SEROEPIDEMIOLOGY OF BLOOD-BORNE VIRUSES AMONG INTRAVENOUS DRUG-ADDICTS IN ISRAEL. M. Dan, M. Rock, S. Bar-Shani. The E. Wolfson Hospital, Holon, and the Central Blood-Bank, Tel Aviv, Israel.

Four-hundred intravenous (IV) drug-addicts were tested for the prevalence of antibody to the human immunodeficiency virus (anti-HIV), and hepatitis B surface antigen (HB_sAg). Other markers of the hepatitis B virus (anti-HB_s, anti-HB_c, HB_eAg and anti-HBe), antibody to hepatitis delta antigen (anti-HD), and antibody to cytomegalovirus (anti-CMV) were randomly determined in part of the study population.

Anti-HIV was found in 8 (2%) IV drug-addicts, and its presence was significantly associated with travel abroad after 1980. At least one marker for hepatitis B virus (HBV) was found in 60% of the examined individuals, while 5.5% (22/400) were carriers of HB_sAg. Five (5.5%) of 90 subjects with HBV markers had anti-HD. Anti-CMV was present in 86.5% of tested sujects.

We conclude that the prevalence of anti-HIV among Israeli IV drug-addicts is much lower than that in the USA and Europe, while the prevalence of HBV markers and anti-HD is not significantly different.

R20.2

EFFECT OF CYCLOSPORINE A AND CYCLOSPORINE G ON INFECTION OF HEALTHY T CELLS BY HIV. M.A. Wainberg, N. Blain, M. Tremblay. Lady Davis Institute, Jewish General Hospital, Montreal, Quebec, Canada.

We have studied the effect of cyclosporine A (CSA) on the ability of HIV to infect healthy peripheral blood lymphocytes (PBL's). To this end, cells were exposed to PHA for 48 hr, after which they were infected by HIV in the presence of polybrene (2 µg/ml). CSA was added to cultures either 2 hr before or at various times after co-incubation with HIV. Infection of cells was assessed by measuring supernatant reverse transcriptase activity and by indirect immunofluorescence assay, using monoclonal antibodies against viral proteins p15 and p24. The use of CSA prior to exposure to HIV completely prevented infection. However, exposure of cells to CSA after co-incubation with virus did not completely prevent infection, although a partial protective effect was observed at times up to 12 hr. Cyclosporine G had no effect in these protocols. CSA did not affect the ability of a HIV-infected cell line, H-9 cells, to replicate virus or to express viral antigens. HIV was able to infect PBL's from allograft recipients on long-term CSA therapy. Lastly, we have succeeded in isolating HIV from each of 12 AIDS patients entered into a small-scale Canadian CSA therapeutic protocol. Supported by Health and Welfare Canada.

R20.3

AFRICAN AIDS IN SOUTH AFRICA. B.D. Schoub, S.F. Lyons, A.N. Smith, S. Johnson, G.M. McGillivray. MRC Aids Virus Research Unit, National Institute for Virology, Private Bag X4,Sandringham 2131, South Africa.

As at the end of 1986, 38 cases of AIDS in South Africans had been notified, all in white males in characteristic Western high-risk groups. In addition, serological surveys in various South African black populations have revealed low prevalence levels (0.1%). Two sentinel cohorts were examined for evidence of African AIDS in South Africa - black prostitutes "servicing" a large industrial complex north-east of Johannesburg (56 subjects) and black women attending the main STD clinic in Johannesburg (195 subjects). Both groups were also investigated for various STDs and found to have prevalence rates consistent with that seen elsewhere in Africa - in the prostitute group 43% positive for HBsAg or anti-HBs and 95% positive for chlamydia antibodies; in the STD cohort 42% positive for HBsAg or anti-HBs, 25% WR positive and 92% positive for chlamydia antibodies. However, with respect to HIV antibodies, none of the prostitute cohort and only one of the STD cohort, a migrant Malawian, were positive, thus confirming that African AIDS has not, as yet, established itself in South Africa.

R20.4

EPSTEIN-BARR VIRUS (EBV) ANTIBODY PATTERNS IN PATIENTS WITH ACQUIRED IMMUNE DEFICIENCY SYNDROME (AIDS). S. Biano (1,3), P. DeGirolami (2,3), D. Hunter (4), N. Mueller (4). (1) Brigham and Women's Hospital, Boston MA 02115, (2) New England Deaconess Hospital, (3) Harvard Medical School, (4) Harvard School of Public Health.

Thirty-one AIDS patients were studied for associations between EBV antibody patterns, and T cell numbers;15 had a primary diagnosis of Kaposi's sarcoma (KS),9 had opportunistic infections (OI), and 7 had both KS and OI (KS+OI). IgM to viral capsid antigen (VCA) was not detected in any of 30 subjects tested. All subjects had IgG to VCA $\geq$ 160(GMT=1309). 21 of 31 (68%) had detectable antibody to early antigen (EA)(GMT=150), and 28 of 31 (90%) had detectable antibody to nuclear antigen (EBNA)(GMT=16). There were no material differences in antibody patterns between the KS group and the OI group, however GMTs of antibody to VCA and EA were lower in the KS+OI group. The absence of IgM, high titers of antibody to VCA and EA, and the presence of antibody to EBNA suggest reactivation/chronic EBV infection. Among 26 subjects who had T cell subset numbers determined, the relation of antibody titers to absolute T cell numbers was complex. Among KS patients antibody titers were positively correlated with absolute T4 cell numbers [Spearman r=0.27;r=0.64(p=0.02); r=0.19; for VCA, EA and EBNA]. Correlations were negative among OI patients [r=-0.52;r=-0.79 (p=0.06);r=-0.03]. In the KS+OI group correlations were positive [r=0.13;r=0.24;r=0.19]

R20.5

PREVALENCE OF ANTIBODIES TO HUMAN IMMUNODEFICIENCY VIRUS (HIV) IN A PENITENTIARY POPULATION. R. Ortiz de Lejarazu, J.M. Eiros, E. Perlado, J.E. Viñuela, A. Orduña, A. Rodríguez-Torres. Department of Microbiology, Faculty of Medicine, University of Valladolid. Valladolid, Spain.

We carried out a seroepidemiological survey of 290 recluses ranging 16-55 years (277 men, 13 women) from Burgos state prison. Sera were assayed with two commercial ELISA (Pasteur and Confirmatory test Abbott) and a Western-Blot test (Lavblot Pasteur).

Antibodies against HIV were present in 25.2% (69 men, 4 women) of the recluses. Prevalence of seropositives by risk groups gave the following percentages: 48.6% (54/111) of I.V. drug abusers and 16.3% (9/55) of homo or bisexuals recluses. A prevalence of 11.5% (17/147) of antibodies was found between recluses not declaring to belong to any of these groups. 30.7% (4/13) of recluses woman were seropositives. Age distribution of seropositives recluses was as follows: 20 (35.7%) of 16-25 years, 49 (25.9%) of 26-35 years, 4 (12.1%) of 36-45 years and none in the 46-55 years group.

These results reflect a similar distribution by risk groups, as described in our country, with a higher antibodies prevalence in the drug abusers group. We found a very high proportion of woman having antibodies against HIV as compared with other surveys.

R20.6

ABSENCE OF HIV AND HTLV-I ANTIBODY IN SERUM SAMPLES OBTAINED IN 1959-1963 FROM INTRAVENOUS DRUG USERS AND CONTROL WOMEN. J.L. Sever, D. Madden, N. Tzan, NINCDS, NIH, Bethesda, MD.

In an effort to determine if HIV or HTLV-I was present in the U.S. during the early 1960s, we examined several sets of serum samples obtained as part of the Collaborative Perinatal Project of the National Institute of Neurological and Communicative Disorders and Stroke, National Institutes of Health, for the presence of HIV and HTLV-I antibody. In this study 345 patients from over 50,000 pregnant women enrolled were selected; 100 patients were normal and represented the major population centers; 53 patients had a history of intravenous drug usage; 35 patients had 10 or more pregnancies; 35 patients had children with erythroblastosis fetalis; 15 patients had advanced carcinoma of the uterus; 13 patients had abnormal cervical epithelium; 21 patients had children who developed malignant neoplasm during the first 4 years of age. Two sera repeatedly tested positive for HIV by ELISA test. One sera reacted in the IFA test on both the infected and control antigen cells. The other was negative on both the infected and control cells. Both samples were negative on Westen blot. One sample was positive in the HTLV-I ELISA and IFA tests. Thus, during the years 1959-1963 none of the individuals had HIV antibody and one had HTLV-I antibody.

R20.7

LAV-2/HIV-2 INFECTION: CLINICAL, EPIDEMIOLOGICAL AND VIROLOGICAL FEATURES. M.Harzic*, M.A.Rey*, S.Gadella**, M.C.Dazza*, F.Brun-Vezinet. Laboratoire de virologie-Hopital Claude Bernard*; Diagnostics Pasteur**. Paris - France.

In 1986, infection by LAV-2/HIV-2 was diagnosed in 18 patients (pts) who have been living in France for at least one year. Six pts presented with AIDS, 2 with ARC and ten were asymptomatic subjects (A.S.). Eleven pts originated from West African countries and 7 pts were europeen: 2 heterosexual Portuguese couples and 3 homosexual French males. In these pts HIV-2 transmission was likely to be acquired through heterosexual or homosexual contacts. HIV-2 exhibited the same lymphotropism and cytopathic "in vitro" effect as HIV-1. Retrovirus isolation was performed in 12 pts from the peripheral blood lymphocytes; virus was detected by RT activity assay in 9 cases (5/5 AIDS; 2/2 ARC and 2/5 AS). Isolates were characterized by dot blot hybridization with HIV-1 and HIV-2 probes. HIV-2 neurotropism was demonstrated by virus isolation from the CSF and/or by intrathecal IgG HIV-2 synthesis. HIV-1 and HIV-2 exhibit cross reactivity restricted to the gag and pol gene products. No cross reactivity was demonstrated between HIV-1 and HIV-2 env gene products. HIV-2 antibodies were detected by Elisa using an HIV-2 purified viral antigen; the presence of antibodies to HIV-2 glycoproteins was demonstrated by Western blot analysis. Cross reactivity with HIV-1 antigens (Elisa) was shown in 24% of the HIV-2 AIDS pts and 60% of HIV-2 paucisymptomatic pts. Specific HIV-2 assays are needed to screen blood donations as to perform HIV-2 seroepidemiological surveys.

R20.8

PROGRESSIVE LEUKOENCEPHALOPATHY OF AIDS: HIV INDUCED PRIMARY DEMYELINATING DISEASE. E.L. Knobler, F.D. Lublin, B.E. Northrup, G. Barolat, S. Duckett, W.S. Robinson. Jefferson Medical College, Philadelphia, PA, USA, Stanford University School of Medicine, Stanford, CA, USA.

Demyelinating disease within the central nervous system (CNS), is a common feature in AIDS. It can range in degree and etiology from progressive multifocal leukoencephalopathy (PML), an extensive demyelinating disease due to an opportunistic papovavirus infection, to small, scattered areas of demyelination for which no cause has yet been found. We report the occurrence of large isolated areas of demyelination in the CNS of two patients with AIDS, which superficially resembled PML. Both presented with progressive ataxia. Biopsies from the cerebellum of each demonstrated widespread demyelination, edema, occasional perivascular cuffs, giant multinucleated astrocytes and abundant foamy macrophages. Ultrastructurally, mature retrovirus particles were observed. Purkinje cell neurons were intact. Oligodendrocyte inclusions, common in PML, could not be found. Hybridization studies demonstrated HIV positivity in these brain tissues, but failed to indicate papovavirus, herpes simplex I, cytomegalovirus or HTLV-I reactivity. These findings thus suggest the possibility that HIV infection may directly lead to demyelination, rather than demyelination occurring as a secondary infection in an immunocompromised host.

R20.9

PERSISTENT ACTIVE VIRAL INFECTIONS OTHER THAN HIV CONTRIBUTING TO THE IMMUNE PATHOGENESIS OF AIDS. G.R.F.Krueger, A.Ramon, M.Seyda, R.Hehlmann, and H.Wolf. Immunopathology Section. Dept. of Pathology University of Cologne, 5000 Cologne 41, W.Germany

A total of 640 patients with LAS, ARC and AIDS were followed immunologically, serologically and bacterio-virologically; specimens included 50 lymph node biopsies for immunohistology and in situ hybridization.
The results show a progressive increase in persistent active EBV (2-80%), CMV (0-95%), HSV (15-45%), and HVB (7.9-14.7%) during the course of the disease, correlating well with the extent of T-cell immune deficiency (T_h cell loss, T_{10} cell increase, β_2 microglobulin, cutaneous anergy). Similarly, the number and extent of bacterial and fungal infection correlated with B-cell and macrophage dysfunction (hyper gamma globulinemia, circulating immune complexes, autoantibodies, defective phagocytosis). The extent of cellular changes in the immune system, however, do not correlate well with the in situ demonstration of HIV DNA or antigen. Thus we propose an interaction of various active infections in the pathogenesis of progressive immune deficiency, which may lead to a vicious cycle of infections and immune deficiency enhancing each other.

R20.10

MOLECULAR PROBING TO MONITOR LYMPHOID ENGRAFTMENT IN AIDS PATIENTS (pts). Keable H*, Bourhis JH*, Lehn P, Vittecoocq D, Rozenbaum W, Vilmer E, Brison O*. Laboratory of Molecular Oncology* CNRS1158 Institut Gustave Roussy and St-Louis and La Pitié Hospitals PARIS - FRANCE.

A pilot trial targetted on AIDS pts is carried out to evaluate the role of lymphocyte transfusions in restoring immunological functions. The purpose of this study rests upon the search of a lymphoid chimera.

7 pts received HPA23 or Suramine followed 21 days later by a total dose of $0,5.10^7$/kg lymphocytes (from histocompatible siblings) biweekly for 3 weeks. No conditionning regimen or ciclosporine were given. Viral and immunological studies were performed concomitantly to molecular monitoring of lymphoid engraftment. Genomic DNA extracted from mononuclear cells of donor and recipient were analysed by Southern blotting prior to transfusions, and monthly thereafter. In case of 2 female recipients whose donor was male probe pY 2.1 specific of Y chromosome was used. In all other cases probing was done using minisatellites (Jeffreys et al, NATURE vol 314 p67, 1985).

Results demonstrate no engraftment in 6 pts. The remaining survivor one shows a possible lymphoid engraftment. He also further distinguishes himself by a negative mixed lymphocytic reaction prior to transfusions and improved immunological functions while viral studies turned negative after treatment.

R20.11

EVOLUTION OF THE HIV SEROPREVALENCE IN GROUPS AT INCREASED RISK FOR AIDS AND GENERAL POPULATION IN SPAIN. R.Nájera, R.de Andrés, J.M. Echevarría, A.Lora A.Tenorio, P.León, J.M.Varela, L.Muñoz, C.Casado and M.Domínguez.Centro Nacional de Microbiología, Virología e Inmunología Sanitarias. Instituto de Salud Carlos III. Majadahonda (Madrid), Spain.

The distribution of the AIDS cases in Spain (242 notified by November 14th of 1986) by groups at increased risk (118 intravenous drug abusers, - IVDA,and 50 men with homosexual practices, HO/BI ♂) is different from the one found in other european countries, except Italy. As the Reference Center for AIDS in Spain we receive samples for confirmation purpose from all the country. In such a way, from January 1st to November 30th of 1986 a study of seroprevalence has been carried out among people belonging to groups at increased risk for AIDS (2424 healthy individuals) and altruistic blood donor (ABD) as general population (133.253 hemodonations). All the samples of serum has been repeatedly tested by several methods of Enzymeimmunoassay (antigen produced in H9 or CEM cells, or by genetic engineering), by Indirect Immunofluorescence on H9/HTLV-III B, H9/FH 1 MN and H9/WF-II cells kindly provided by Dr. R.C.Gallo (National Institutes of Health, Bethesda) and on CEM clon 13/LAV and Molt 4/LAV cells sent by Dr. L.Montagnier (Pasteur - Institut, Paris) as a generous gift, and if there was no agreement in results also by Immunoelectrotransference/"Western Blot". The results show a 65.3% HIV seropositivity among IVDA (very high), 50.3% among haemophiliacs (also very high), 20.8% among HO/BI ♂ (a relatively low level of infection) and - 0.08% among ABD. In 1985 the respective rates were 63.6%, 68.3%, 12.8% - and 0.08%.

Data related to other exposed populations (health care workers, female prostitutes, heterosexual sex partners and children born from persons at increased risk for AIDS and LAV-2 seroprevalence are also presented.

R20.12

INVESTIGATION OF HIV CONFIRMATORY TESTS ON SERA OF AMAZONIAN ABORIGENES. M. Fauvel[1], J.C. Soto[2], L. Guevara[3], A. Colichon[3]. Laboratoire de santé publique du Québec[1], D.S.C. Hôpital St-Luc[2], Montréal, Canada, Univ. Péruvienne Cayetano Heredia[3], Lima, Pérou.

Ninety-two sera collected since 1975 from Amazonian aborigenes and found positive in an EIA assay were investigated by immunofluorescence (IFA), radioimmunoprecipitation assay (RIPA) and Western blot (WB) to confirm HIV seropositivity. Seventy-one of these sera were found reactive by RIPA. Titers ranged from undiluted to 100. Thirteen RIPA positive sera were also reactive by WB.

When 51 of the RIPA positive sera were analyzed by IFA, 49 revealed uncharacteristic fluorescence on all LAV-infected and non-infected MOLT-4-T4 cells. However, two sera revealing high titers in RIPA also showed titers of ⩾ 320 and 80 respectively in IFA.

When selected weak reactors were treated with heparin-$MnCl_2$, reactivity disappeared in 1 of 9 sera tested by RIPA. Treatment of the two high titer sera with anti-human IgG eliminated reactivity with HIV-polypeptides.

These results show that sera frozen since 1975 may give non-specific reactions by RIPA and WB. However, two of the sera collected from Amazonian aborigenes revealed antibody-mediated reactions. The meaning of these reactions remains to be elucidated.

R20.13

PRODUCTION OF A HIV-1 POSITIVE REFERENCE ANTISERUM FOR WHO. H.Hampl, K.-O.Habermehl, B.Zorr, H.Zeichhardt. Institute for Clinical and Experimental Virology, Free University of Berlin, Germany.

A batch of 10000 ampules containing 200 µl lyophilized antiserum each has been prepared from one patient. The patient showed no clinical symptoms or illness. The serum was inactivated for 60 min. at 56° C and thereafter diluted 1:20 in hypotonic phosphate buffer, it was HBsAg-negative, and showed no "stickyness" after heat-inactivation. A good reactivity could be observed with a number of ELISAs and in Western blot strips of different origin.

On behalf of the World Health Organization an international study was performed at the National Institute for Biological Standards and Control (London) by Dr.G.Schild and Dr.J.Garrett in cooperation with 21 other laboratories who confirmed the usability of this standard (publ. in WHO-Bulletin, in preparation).

R20.14

COMPARATIVE QUANTITATIVE INVESTIGATIONS ON HIV-1-NEUTRALIZING ANTIBODIES WITH RESPECT TO DIFFERENT EPITOPES. B.Zorr & K.-O.Habermehl. Institute for Clinical and Experimental Virology, Free University of Berlin, Germany.

Quantitative determination of HIV-neutralizing antibodies is a prerequisite for developing vaccines. Using a sensitive plaque reduction assay 50 sera of HIV-1-infected patients showed a linear correlation between HIV-specific and neutralizing antibodies. Since all sera showed this correlation one can conclude that in spite of the genetic variability of HIV-1 the majority of the neutralizing epitopes seems to be conserved. This indicates that similar as in Hepatitis B the total amount of (ELISA-determined) HIV-1-antibodies gives an information about the humoral immune status of the patient. - Further investigations concerning single epitopes on different structural proteins have been performed using synthetic oligopeptides. One epitope (on p18) showed a significant concentration-dependent correlation between ELISA-reactivity (based on this epitope) and its neutralizing activity. Since all sera (n=30) showed this behaviour one may conclude that this epitope has a high genetic stability. Similar results could be obtained with an epitope from gp 41 whereas another epitope on gp 41 showed no genetic stability. The results were confirmed by antisera obtained from sheep immunized against the corresponding oligopeptides (synthesis of the oligopeptides and immunizations were done by Dr.Frenzel, Biochrom).

R20.15

HUMAN IMMUNODEFICIENCY VIRUS (HIV) REVERSE TRANSCRIPTASE (RT) INHIBITING ANTIBODY IN HIV SEROPOSITIVE HOMOSEXUAL MEN. K. Sano, M. Lee, F. Callegari, M. Advani, K. Sugita and D.T. Imagawa. Department of Pediatrics, Harbor-UCLA Medical Center, Torrance, CA, U.S.A.

Our study shows that 33 out of 49 (67%) of HIV seropositive, asymptomatic men produce RT inhibiting (RTI) antibody. Such an inhibitory antibody has been described for only a few retrovirus infections. Our data show that the RTI antibody to HIV is very specific. The specificity was demonstrated by kinetic studies and lack of inhibition to the RT of other retroviruses and DNA-dependent DNA polymerase. The results also show a strong correlation between the presence of RTI antibody and the lack of viremia in HIV seropositive men. HIV was isolated from peripheral blood lymphocytes in only 18% (6/33) of RTI antibody positive men. In contrast, 63% (10/16) of RTI antibody negative men yialded HIV. As expected, 39 seronegative men showed no RTI antibody and no virus was isolated. These findings suggest that this antibody may interfere with virus replication. Another possibility is that this antibody may be a consequence, and thus an indicator, of the state of virus infection in its host. Whatever may be the case, further examination is needed, and may prove to be of diagnostic, prognostic, or therapeutic value in the study and treatment of AIDS.

R20.16

EPSTEIN-BARR VIRUS (EBV) EXCRETION DETERMINED BY NUCLEIC ACID HYBRIDIZATION IN HOMOSEXUAL AND HETEROSEXUAL MALES. F. Diaz-Mitoma, S. Houston, B. Romanowski, A. Ruiz, D.L.J. Tyrrell. Department of Medical Microbiology and Infectious Diseases, Faculty of Medicine, University of Alberta, Edmonton, Alberta, Canada.

EBV has been associated with several complications including lymphomas in AIDS patients. We studied the excretion of EBV in heterosexual and two groups of homosexual males. Volunteer gay men had a complete history and physical examination. Controls recruited from the STD clinic were heterosexual males with no AIDS risk factors. Throat washings were obtained from all subjects. The DNA extracted from pharyngeal cells was hybridized with the Bam H1-W fragment of the EBV genome. Thirty two (60%) of 52 gay men and 14 (30%) of 46 heterosexual males were excreting EBV ($p = < 0.01$). Eighteen or 78% of 23 gay men with abnormal lymphadenopathy or systemic symptoms of ARC or AIDS and 13 or 44% of 29 asymptomatic gay men were excreting EBV ($p = 0.01$). The level of EBV excretion in homosexual males was higher than in heterosexual controls.

The higher prevalence and level of EBV excretion in homosexual than in heterosexual males could be explained by immunosuppression or by a higher risk of EBV exposure in gay men. EBV might contribute to the symptoms in homosexual males. More likely EBV reactivation is enhanced by immunosuppression due to human-immunodeficiency-virus infection.

R20.17

SEROLOGICAL DIAGNOSIS AND EPIDEMIOLOGY OF HIV INFECTIONS IN FRANKFURT, AN AIDS ENDEMIC AREA OF WESTERN GERMANY. H.W.Doerr, T. Klenk, K. Mergener.
Department of Medical Virology, Centre of Hygiene, University Clinics of Frankfurt, FRG.

Basing on screening ELISAs for serum antibodies to HIV during 1985 and 1986, the monthly attack rate of infection was estimated to be at least 50 persons in the area of Frankfurt/FRG. The majority of them belonged to risk groups. Female prostitutes were found seropositive in about 2.5%. The first case of infection with HIV II was detected in December1986. The clinical examination of HIV infected patients admitted to an usual City Hospital demonstrated signs of LAS/ARC in about 40%, of full blown AIDS in about 6%. Western blot analysis of serum antibodies revealed a restriction mainly to IgG 1 (present in 100% of persons investigated) and IgG 3 (additionally present in about 50%). Polypeptide patterns seen with CSF specimens were restricted to IgG 1 and differed sometimes from them of corresponding serum antibodies. Specific serum or CSF IgM was rarely, IgA not detected in any case. The ELISA for HIV antigen was positive in about 60% of AIDS patients.
Besides HIV, the results of herpesvirus (CMV, EBV, HSV, VZV) and hepatitis B serology were evaluated.

R20.18

RIBAVIRIN DELAYS PROGRESSION OF THE LYMPHADENOPATHY SYNDROME TO THE ACQUIRED IMMUNE DEFICIENCY SYNDROME. PAA Mansell, RB Roberts, PNR Heseltine, GH Dickinson, JM Leedom, RM Johnson. University of Texas, Houston; Cornell University, NY; University of Southern California, Los Angeles; University of Miami, Miami.

Ribavirin, a synthetic guanosine nucleoside analogue has in vitro activity against the human immunodeficiency virus (HIV). Ribavirin's role in preventing progression of the lymphadenopathy syndrome (LAS) to AIDS was evaluated in a randomized, double-blind, placebo-controlled, multicenter trial. One hundred and sixty-three homosexual men with LAS were treated with oral ribavirin or placebo for 24 weeks, followed by no treatment for four weeks. All were HIV culture positive, had lymphadenopathy for six months or more, hematocrits $\geq$ 35% and fewer than 500 ($\pm$ S.D. of the method) T4+ cells. Those with chronic symptoms of HIV infection (diarrhea, fever, thrush, weight loss) were excluded. Fifty-two received 800 mg of ribavirin daily and none developed AIDS; 6/55 (11%) given 600 mg/day of ribavirin and 10/56 (18%) taking placebo developed AIDS ($p=0.007$). Difference in outcome correlated with ribavirin plasma levels. This therapeutic effect was not explained by differences in T4+ cells or hematocrit at initiation of therapy. Qualitative HIV cultures remained positive and measured immunologic function did not increase in the treated groups. Three of 107 (2.8%) receiving ribavirin discontinued treatment because of insomnia or nausea/vomiting. Ribavirin was associated with a mild compensated anemia; no one required a blood transfusion. There was one death, AIDS-related, in the placebo group. Prolonged ribavirin therapy is well tolerated and delays the progression to AIDS of immunologically compromised men with LAS. Ribavirin deserves further study as a therapeutic agent for HIV infection.

R20.19

Incidence and persistence of virus colonization in homosexual volunteers seropositive and seronegative for human immunodeficiency virus (HIV). MICHAEL LANDE, E.D.KLEIN, D.L.PERRO, D.McKINLEY, L.Z.COOPER, H.H.BRIEGS. St. Luke's-Roosevelt Hospital Center, Columbia University, New York, New York.

A high incidence of infection with cytomegalovirus (CMV) and herpes simplex (HSV) has been described in patients with AIDS and ARC and has been ascribed to reactivation as a result of the underlying retrovirus induced immunodeficiency.

We have followed a cohort of 109 homosexual volunteers since 1981-82 for 3 years. 44 were HIV seronegative (HIV neg), 60 seropositive (HIV pos), and 5 have seroconverted for a total of 65 seropositives. 32 have developed ARC/AIDS.

Samples of heparinized blood, urine, semen, rectal swabs (RS) and throat gargles (TG) taken from the volunteers were cultured for presence of viruses at 6 monthly intervals. Samples from 33 heterosexual men were also cultured for virus presence. CMV and Enteroviruses (Entero) were isolated in 75 and 57 volunteers, respectively, and HSV in 16. Except for adenoviruses which were isolated from 11/63 (17.5%) HIV pos and from 3/44 (7%) HIV neg individuals, no significant difference in isolation rate for CMV, Entero, and HSV was noted between the HIV pos and HIV neg groups. CMV persisted for >18 months in 18 HIV pos and in 11 HIV neg volunteers. Blood cultures were positive for CMV in 8 (12.3%) HIV pos and in 5 (11.4%) HIV neg, and for Entero in 3 (4.6%) HIV pos and 5 (11.4%) HIV neg volunteers. Persistence of CMV did not correlate as a predictor for development of ARC/AIDS in HIV pos subjects, whereas a virtual absence of Entero was noted in those patients who progressed to AIDS. Of 33 controls, only 3 were positive for Entero in rectal swabs.

Virus colonization with CMV, Entero, HSV, and Adenoviruses is a very frequent occurrence in homosexual men living in New York. The incidence of these virus infections appears to be independent of the states of HIV infection. (Grant NIH/NIAID IRO1A 121175-03)

R21.1

ETUDE COMPARATIVE DE LA PERSISTANCE DU VIRUS MORBILLEUX SUR DEUX SYSTEMES CELLULAIRES. F.Z. Sbih, K. Kourouma, A. Bouguermouh, A. Chikhi. Département de Biologie moléculaire, Faculté des Sciences de la Nature, Université U.S.T.H.B, Alger, ALGERIE.

La persistance du virus morbilleux a été établie sur deux systèmes cellulaires : les cellules VERO et les cellules McCoy. L'infection persistante sur cellules VERO a été obtenue par passage répété du virus non dilué. Elle a été maintenue jusqu'à présent après 65 passages pendant 4 années. Les cellules McCoy se sont par contre révélées peu permissives au virus morbilleux. Elles ont subi jusqu'à présent 52 passages. L'hémadsorption et l'immunofluorescence ont permis d'observer que la majorité des cellules VERO et cellules McCoy infectées de façon persistante contenait les antigènes viraux au niveau de leur membrane cellulaire. Les deux systèmes cellulaires infectés libèrent du virus infectieux dans le surnageant. Sur cellules VERO, la production du virus infectieux diminue avec le nombre de passages. Pour les mécanismes, deux de nos résultats suggèrent l'existence de particules défectives interférantes dans le système VERO alors que nous avons décelé une activité antivirale dans le surnageant des cellules McCoy infectées de façon persistante.

R21.2

EFFECT OF ACUTE CYTOMEGALOVIRUS INFECTION ON BLOOD COAGULATION OF NEWBORN GUINEA PIGS. Z.M.Zheng, M.Lin, J.H.Zhang, W.P.Zhu, J.M. Hu. Virus Research Institute, Hubei Medical College, Wuhan, P.R.China.

Previous study has shown that platelet counts could be reduced due to acute cytomegalovirus (CMV) infection. In present study, Sc. inoculation of 28 newborn guinea pigs (GP) with 1 ml of 5.5 log10 TCID50 of GP cytomegalovirus (GPCMV) resulted in acute CMV infection. Infectious CMV was recovered from the salivary glands of infected GP since 5 day postinfection. GP infected developed growth retardation, salivary gland swelling, and splenomegaly. Histological exam found many virus inclusions in the salivary duct cells and necrosis foci and sinal acute catarrhal inflammation in the spleen.

Compared to age-matched controls, platelet counts were significantly lower in infected GP and prothrombin time of infected GP was 6 times longer. moreover, 18/26 of infected GP had abnormal clot retraction, but only 6/22 of controls did. Using an immunological latex agglutination test, serum FDP levels of controls are <3.2 μg/ml, whereas infected GP had a serum FDP level respectively 11.2, 9.0, 6.4, 6.4 μg/ml at 6, 9, 12, 16 days postinfection. Therefore, the study provided the evidence that acute CMV infection of newborn GP could disturb normal blood coagulation.

R21.3

THE LOCAL ORIGIN OF FEVER IN INFLUENZA. C.M.Tinsley, C.Sweet, D.M.Coates, H.Smith. Department of Microbiology, The University of Birmingham, Birmingham B15 2TT, U.K.

In influenza of ferrets, which provides a good model for the human disease, fever results from virus interaction with phagocytes locally in the upper respiratory tract (URT). Following nasal infection with a virulent virus (7a), onset of fever correlated with influx of nasal inflammatory cells. Many of these cells contained virus antigen and, when incubated in vitro, released a pyrogen with properties of EP. Insufficient virus escaped into the bloodstream from the URT to produce the observed fever by interaction with phagocytes in the RES.

If fever is local in origin then nasal phagocytes harvested during the pyrexial period from ferrets infected with viruses exhibiting different levels of fever, should release, on incubation in vitro, EP at levels commensurate with the observed in vivo fevers. Another virulent virus (64c) produced a high fever during infection and nasal exudate cells from such febrile ferrets released greater quantities of EP than 2 attenuated viruses (A/Fiji, A/PR/8) which produce low fevers during infection. Two other viruses (64d, A/USSR) produced moderate fevers during infection and the levels of EP released by phagocytes were intermediate between that of the virulent (7a, 64c) and attenuated (A/Fiji, A/PR/8) viruses.

R21.4

SUPPRESSION OF NK ACTIVITY OF SPLEEN CELLS BY CHICKEN FETAL ANTIGEN PRESENT ON MAREK'S DISEASE LYMPHOBLASTOID CELL LINE CELLS. T. Mikami, K. Ohashi, T. Fujieda, H. Kodama, H. Izawa. Department of Epizootiology, Faculty of Veterinary Medicine, Hokkaido University, Sapporo 060 Japan.

Chicken fetal antigen (CFA) expressed on the cell surface of Marek's disease (MD) lymphoblastoid cell line (MDCC-MSB1) cells was purified by affinity chromatography using a monoclonal antibody (2H3). A 4-hour ^{51}Cr release assay was performed using spleen cells as effector cells to investigate the role of the CFA in the immune response of chickens, especially in relation to natural killer (NK) activity.

Spleen cells of specific-pathogen-free (SPF) chickens showed reduced NK activity in the presence of CFA in vitro, as did those cells treated with MSB1 soluble antigen. Furthermore, the NK activity of spleen cells from chickens treated with CFA was also suppressed when compared to the cells from untreated or ovalbumin treated chickens. In addition, MSB1 clo. 18 transplantable tumors grew progressively in some of the 14-day-old chickens treated with CFA, whereas the tumors regressed in age-matched chickens with or without treatment by ovalbumin.

R21.5

EXPERIMENT INFECTION WITH A PANDEMIC STRAIN OF RINDERPEST VIRUS ISOLATED FROM A WILD BUFFALOE.

Dr.R. Nawathe and W.P. Taylor* University of Maiduguri, Nigeria and *Animal Virus Research Institute, Pirbright, England.

The devastating 1983-85 panzootic of rinderpest took a toll of over 3 million cattle in central and West Africa. The disease not only affected cattle but also a number of wild ungulate species like the buffaloe, eland, bush buck, water buck, duiker, kob, wathog, bush pig etc. However, domestic sheep and goats were not affected.

An isolate of the rinderpest virus from a wild buffaloe was inoculated into 4 steers. The average incubation period worked out to be 4.25 days followed by fever for 3.25 days, diarrhea and death in further 4 days. The symptoms and lesions indicated that the virus strain was virulent. Inoculation of the strain into local sheep and goats did not produce any clinical signs but seroconversion was observed.....

R21.6

MECHANISM OF VIRAL CLEARANCE: GENERATION OF VIRUS SPECIFIC CYTOTOXIC T CELL RESPONSE BY "TOLERANT" MICE FOLLOWING IMMUNE THERAPY. B. D. Jamieson & R. Ahmed, Microbiology & Immunology UCLA School of Medicine, Los Angeles, CA 90024.

Infection of mice with lymphocytic choriomeningitis virus (LCMV) is a classic model for studying viral persistence and antigen specific T cell unresponsiveness. Mice infected at birth or in utero with LCMV become life-long carriers with no detectable LCMV specific T cell responses. Adoptive transfer of lymphocytes from immune mice results in reduction of infectious virus and viral antigen from carrier mice. This clearance is mediated by LCMV specific MHC class I restricted $Lyt2^+$ T cells. Using congenic mice (Thy 1.1 & Thy 1.2) we were able to distinguish between activity due to immune donor T cells and carrier host T cells. We have found that: 1) Transferred T cells can be recovered from the carrier host 8 months after immune therapy. These long-lived T cells are capable of expansion and viral clearance upon a second transfer in vivo and exhibit specific killing of LCMV infected cells in vitro. 2) In vivo depletion of donor T cells results in respread of the virus in 40% of treated mice, showing that in these mice, donor T cells were responsible for controlling the infection. 3) The remaining 60% of treated mice show no viral respread and can generate a host derived LCMV specific CTL response. These mice are able to resist a second challenge of LCMV demonstrating the acquisition of immunocompetence by a host previously unresponsive to the virus.

R21.7

REPLICATION OF HSV-1 IN MURINE PERITONEAL MACROPHAGES IS DEPENDENT ON VIRAL INTRAPERITONEAL PATHOGENICITY SEQUENCE AND PREINCUBATION OF CELLS WITH L-CELL CONDITIONED MEDIUM. T. Ben-Ruc*, G. Darai** and Y. Becker*. *Department of Molecular Virology, Faculty of Medicine, The Hebrew University, Jerusalem, Israel. ** Institute for Medical Virology, University Heidelberg, Heidelberg, West Germany.

The pathogenicity of HSV-1 strains injected into mice by the intraperitoneal (IP) route was compared to their ability to infect peritoneal macrophages (mφ) under *in vitro* conditions. Virulent HSV-1 strains F and KOS caused cytopathic effect (CPE) in peritoneal mφ from susceptible mouse strain A, but not in mφ from resistant mice C57BL/6. In spite of the CPE, virus replication was not possible. Preincubation of mφ with L-conditioned medium (LCM) containing mφ specific, colony stimulating factor (CSF-1) obtained from Prof. R. Gallili (Dept. of Immunology, our medical school) made possible virus replication, depending on the length of exposure of mφ to the growth factor. In the pretreated mφ, HSV-1 strains F and KOS produced the immediate-early (IE) mRNAs, followed by replication of viral DNA. A rise in virus titer between 6 and 24 hr p.i. was demonstrated. The IP apathogenic HSV-1 strain HFEM, which lacks the HpaI P sequence, was unable to cause CPE in mφ or to express the IE genes, nor were viral DNA or progeny produced. It is concluded that the HpaI P sequence is responsible for the recognition of HSV-1 by peritoneal mφ.

Supported by U.S.-Israel Agricultural Research and Development Fund - BARD, The Foundation for the Study of Molecular Virology and Cell Biology, Arizona, and The Foulkes Foundation, U.K.

R21.8

ROLE OF LANGERHANS CELLS (LC) AND SPLEEN CELLS IN THE TRANSFER TO NEWBORN MICE OF GENETIC RESISTANCE TO HSV-1 SKIN INFECTION

E. Sprecher and Y. Becker, Department of Molecular Virology, Faculty of Medicine, The Hebrew University, Jerusalem, Israel.

LC which function as highly efficient ATPase$^+$ and Ia$^+$ antigen-presenting cells in the epidermis play an essential role in the defense of the skin against HSV-1 infections (Sprecher & Becker, Arch. Virol. 91:341, 1986). Suckling mice are known to be far more susceptible to intraperitoneal HSV-1 infection than adult mice. We also found them to be highly sensitive to HSV-1 skin infection in comparison with adult mice. Because the immune response to HSV-1 skin infection is known to be mainly cellular, the antigen-presenting and the immune-effector functions following HSV-1 skin infection of newborn mice, were studied. Staining whole epidermal mounts revealed numerous Ia$^+$ and ATPase$^+$ cells in the epidermis of suckling mice and adult mice. LC function was studied in a skin lymphocyte reaction assay in which newborn-derived LC were shown to stimulate adult-derived T cell proliferation to the same degree as adult-derived LC. A severe defect in the newborn-derived T-cell responses was observed, suggesting that T-cell deficiency is responsible for the higher susceptibility of newborns to skin HSV-1 infection. To test this idea, HSV-1 was coinjected with various preparations of adult-derived spleen cells devoid of antigen-presenting cells. Only in the resistant C57BL/6, and not in the sensitive A and Balb/c strains, did the co-injected lymphocytes confer protection against HSV-1 infection. Thus, genetic resistance can be transferred from adult mice to suckling mice by spleen cells. The nature of the lymphocyte subset involved in the transfer of genetic resistance is under study. Supported by the U.S.-Israel BSF, The Foundation for the Study of Molecular Virology & Cell Biology, Ariz., and The Foulkes Foundation, U.K.

R21.9

LEUKOCYTE IDENTIFICATION IN VIRUS-INDUCED MYOCARDITIC LESIONS BY IN SITU IMMUNE AUTORADIOGRAPHY. E. Godeny and C. Gauntt. Department of Microbiology, University of Texas Health Science Center at San Antonio, U.S.A.

Coxsackievirus B3 (CVB3) induces myocarditis in murine models with features similar to that found in humans; however, the cells involved in the inflammatory lesions have yet to be identified. An in situ immune autoradiography procedure was developed for this purpose. CVB3 capsid antigens were detected in murine heart tissues but not in myocarditic lesions. Virus-induced antigen(s) were also detected by binding of the lectin, Ulex europeaus agglutinin I to heart tissues at the time of maximum CVB3 capsid antigens detection. B cells were not detected. T cells (effector and helper) were identified within cardiac lesions by binding of monoclonal antibodies to Thy1$_2$, Lyt1, Lyt2, and L3T4 surface markers. Macrophages (Mac1$^+$) were detected early during lesion formation but were absent from heart tissues after day 7 of infection. Antibody to asialo Gm$_1$ reacted within myocarditic lesions, suggesting the presence of natural killer cells. Asialo Gm$_1$-sensitive cells with lytic activity for YAC-1 cells were isolated from mechanically-disrupted hearts of infected mice. These data suggest that CVB3-induced myocarditic lesions contain macrophages, effector and helper T cells and natural killer cells.

R21.10

DEMONSTRATION OF NATURAL KILLER CELL-MEDIATED LYSIS OF BOVINE VIRAL DIARRHEA VIRUS-INFECTED CELLS. D.M. Justewicz, R. Magar, and J. Lecomte. Virology Research Center, Institut Armand-Frappier, Laval, Québec, Canada H7N 4Z3.

Studies were undertaken to determine if infection with bovine viral diarrhea virus (BVDV) rendered MDBK cells more susceptible to natural killer (NK) cell-mediated lysis. Two approaches were used. (i) Analysis of the kinetics of bovine NK activity against the MDBK epithelial cell line - used as target cell in a chromium-release assay - showed it to be insensitive, even after 16 h. In contrast, BVDV (NADL)-infected MDBK cells were markedly sensitive to NK activity, which progressively increased with time, up to 40 ± 2.2% at 16 h, with an effector: target ratio of 100:1 and with MDBK infected at a multiplicity of infection (moi) of 1.0 for 16 h prior to NK assay. Lower E:T ratios resulted in lesser NK activity, and the relationship between effector cell number and NK activity was linear. (ii) The NK response against BVDV-infected cells was selective, since it was dependent on the moi used. For example, at 5.0 moi NK activity was increased by almost two-fold over that for 1.0 whereas 0.1 moi decreased it, by 2.5-fold. NK activity was shown to correlate with the percentage of infected cells, as indicated by indirect immunofluorescence with BVDV-specific antiserum. However, maximum NK activity against BVDV-infected cells was about five-fold less for the prototypic NK-sensitive K562 target cell. These results suggest that BVDV is NK-sensitive, and favor a role for NK cells in a nonspecific immune response to BVDV infection.

R21.11

REASSORTMENT OF THE GENOME SEGMENTS OF BLUETONGUE VIRUS IN CULTURED CELLS, THE VERTEBRATE HOST, AND THE VECTOR. R.F. Ramig[1], S.K. Samal[1], A. El-Hussein[2], D-Y. Chen[1], F. Holbrook[3], B. Beaty[2], C.W. Livingston[4], and S. McConnell[5]. Dept. of Virology[1], Baylor College of Medicine, Houston, TX; Dept. of Veterinary Microbiology[2], Colorado State University, Ft. Collins, CO; USDA Arbovirus-borne Animal Disease Research Laboratory[3], Laramie, WY; Texas Agricultural Experiment Station[4], San Angelo, TX; Dept. of Veterinary Microbiology, Texas A&M University[5], College Station, TX, USA.

Mixed infections of Vero cells, sheep, and *Culicoides variipennis* were performed with culture-adapted strains of bluetongue virus (BTV) serotypes 10 and 17. Random progeny from mixed infections were selected at various times postinfection, grown to high titer and the parental or reassortant electropherotype of each clone was determined by electrophoresis.

72 (48%) of 203 progeny clones isolated from mixedly infected Vero cells were reassortant. Furthermore, reassortment was an early event with reassortants detected as early as 12 hrs and increasing to a plateau of 60-75% by 24 hrs. 16 (5%) of 310 progeny clones isolated from two mixedly infected sheep were reassortant, with reassortants being isolated between 1 and 9 days postinfection. 75 (42%) of 180 progeny clones isolated from *C. variipennis* were reassortant, although the frequency varied greatly from fly to fly (7-78%).

In each mixed infection, one parent dominated the infection and contributed the majority of genome segments to reassortant progeny. However, in each case preferential selection of certain genome segments from one parent appeared to occur.

R21.12

IN SITU CHARACTERIZATION OF INFLAMMATORY CELLS IN THE SKIN AND DIGESTIVE TRACT OF CATTLE PERSISTENTLY INFECTED WITH BVDV. H. Bielefeldt Ohmann, Dept. Vet. Virol. & Immunol., The Royal Vet. and Agric. Univ. of Copenhagen, Denmark.

The phenotypic identity of mononuclear leukocytes in tissues from BVDV-infected animals were examined using sensitive single- and double-labelling immunohistochemical techniques. Occurrence and distribution of IgM$^+$ (B-) cells, T cells of either the helper or suppressor/cytotoxic subsets and MΦ was investigated. No difference was detected between clinically normal, infected calves and uninfected control subjects, despite widespread virus-antigen presence in a variety of cells in viraemic animals. MΦ and Langerhans cells (LC) were prevailing cell types in the keratinized epithelial and suepithelial connective tissues, with occasional T-lymphocytes (mostly intraepithelial and of the BoT8 phenotype). No B-cells were observed. Some of the infiltrating cells also contained virus antigen. In animals suffering from and succombing to Mucosal Disease, the hyper- and parakeratotic changes, as well as necrotizing exanthemas were accompanied by massive infiltration in dermis and epithelium of Ia-positive cells (MΦ and possible LC), some T-cells (predominantly BoT8$^+$ cells) and only rarely B-cells. Some of the infiltrating MΦ stained with antibodies specific for activated MΦs. These latter cell populations also infiltrated the l.propria of the gut. Taking into account the recent results on the biology of the virus and the immunotolerant stage of the animals, a hypothesis for the mechanism(s) involved in the development of pathological lesions and clinical disease is presented. (Funded by SJVF 13-3681).

R21.13

GENOMIC DIFFERENCES BETWEEN DIABETOGENIC AND NON-DIABETOGENIC VARIANTS OF ENCEPHALOMYOCARDITIS VIRUS. Ji-Won Yoon, Alex K.C. Wong and H. Myung Eun. Department of Microbiology and Infectious Diseases, Faculty of Medicine, University of Calgary, Calgary, Canada.

The M variant of encephalomyocarditis (EMC) virus can induce diabetes-like syndrome in mice by infecting and destroying pancreatic beta cells. Plaque purification of M variant of EMC virus resulted in the isolation of two stable variants: one diabetogenic (EMC-D) and the other nondiabetogenic (EMC-B). EMC-D and EMC-B could not be distinguished antigenically. However, oligonucleotide fingerprinting after T1-digestion of the RNAs from these two variants revealed that an oligonucleotide was missing from the RNA of EMC-B but was present in the RNA of EMC-D. The oligonucleotide unique to EMC-D was sequenced as 5'ACAAUCUCACUUUUCCAACAACAAG3'. Molecular hybridization studies using a DNA primer complementary to the EMC-D specific RNA sequence and other studies revealed that the absence of the corresponding spot in EMC-B appears to be due to a point mutation rather than a deletion. This point mutation identified as "U" to "C" transversion at the 3' end of the oligonucleotide could introduce an amino acid change from Ser to Ala at the I and F proteolytic cleavage site. It is concluded that the EMC-D viral genome differs from the EMC-B viral genome by at least a point mutation that may influence the pathogenesis of EMC virus-induced diabetes.

R21.14

INFECTION OF HUMAN BLOOD MONONUCLEAR CELLS BY MEASLES VIRUS STRAINS. J. Ilonen, R. Salonen, R. Marusyk, A. Salmi. Nat. Publ. Hlth. Inst., Oulu, Finland, Dept. Med. Microbiol. & Inf. Dis., Univ. Alberta, Edmonton, Canada.

Eight measles virus strains including several SSPE isolates were compared as to their growth characteristics in human peripheral blood mononuclear cells. The Edmonston strain virus and some other strains replicated in PHA-stimulated lymphocytes and released high titers of infectious virus into the culture. Inhibition of lymphocyte proliferation was relatively low at the beginning of DNA synthesis and could be detected only at high multiplicity of infection. At 3-4 d after initiation of culture, a strong inhibition was seen also in cultures infected with low doses of virus, and was apparently related to the production of high titers of new infectious virus.

Some virus strains originally isolated from SSPE patients produced only a small amount of infectious virus in lymphocytes at 37°C but had a very strong inhibitory effect on lymphocyte proliferation leading to intensive syncytia formation and cell death within three days.

The finding of differences in the characteristic of lymphocyte infection suggests that the effect of measles infection on the immune regulation may also be dependent on the particular strain involved. The nature of the initial virus-lymphocyte interaction may determine the outcome of infection and lead to chronic sequelae in some cases.

R21.15

HUMAN CYTOMEGALOVIRUS (HCMV) REPLICATES IN PRIMARY HUMAN BONE MARROW CELLS AND T LYMPHOCYTES

R. W. Braun, H. Reiser, and J. Kühn
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Persistent infections of human leukocytes with human cytomegalovirus (HCMV) have been known for many years. As an attempt to further elucidate the pathogenesis of HCMV infection we have studied the replication of HCMV in primary human bone marrow cells (BMC) and peripheral blood T cells. Whereas freshly isolated BMC and T cells were resistent against HCMV infection, T cells grown in the presence of Interleukin-2 and BMC precultivated for three weeks allowed replication of progeny virus. This was documented by titer rises, by expression of viral early and late antigen as assessed by immunofluorescence, SDS-PAGE, and Western-blot and by determination of viral DNA synthesis. As determined by infectious center assays only a subset of each of these cell cultures allowed replication of HCMV. By further experiments on the nature of the viral target cell it could be shown that as well $T4^+$ and $T8^+$ cells as bone marrow fibroblasts and bone marrow stem cells allowed replication of HCMV. Furthermore, in BMC a short time persistence of HCMV infection was observed, which may suggest a side of viral persistence in BMC. From the summary of our data we conclude that bone marrow fibroblasts, bone marrow stem cells and human peripheral blood T cells are targets for HCMV infection under appropriate conditions.

R21.16

The role of interferon-γ in clearance of lymphocytic choriomeningitis virus from mice. L.S. Klavinskis and M.B.A. Oldstone. Department of Immunology, Research Institute of Scripps Clinic, La Jolla, CA, USA.

Previous studies have shown that adoptive transfer of cloned cytotoxic T lymphocytes (CTL) that are lymphocytic choriomeningitis virus (LCMV) specific and H-2 restricted into mice infected with LCMV-Armstrong (which acutely infects adult mice) reduces their splenic virus titre by 99% in 24 hours.. Here we address the issue whether virus specific CTL's in addition to direct cytolysis may exert their antiviral effect in vivo by release of lymphokines with direct/indirect antiviral activity. Using recombinant murine interferon-gamma (IFN-γ) we show that splenic virus titre 48 hours post-infection is not significantly reduced if IFN-γ (range 10^4 to 4×10^6 units) is given intravenously either 1 hour before, or at 12 hour intervals post inoculation. Similarly intrasplenic injection with IFN-γ 1 hour before inoculation with virus failed to reduce splenic virus titre. However, prophylactic injection of 10^5 units, 24 hours prior to infection with LCMV-Armstrong resulted in 99% reduction in splenic virus titre 48 hours post infection. Other evidence for a role of IFN, in vivo, was the ability to abrogate a persistent infection in adult mice with LCMV-Clone 13 (a genetic variant of LCMV-Armstrong which causes a persistent infection in adult mice) by treatment with IFN-γ 24 hours before virus inoculation.

R21.17

STATUS OF INSULIN SYNTHESIS AND CELLULAR mRNA IN PANCREATIC ISLETS OF COXSACKIEVIRUS B4-INDUCED HYPERGLYCEMIC MICE. N. K. Chatterjee, C. Nejman, I. Gerling. Wadsworth Center for Laboratories and Research, New York State Department of Health, Albany, N.Y. 12201 U.S.A.

Infection of mice with diabetogenic strains of coxsackievirus B4 produces a diabetic syndrome resembling human juvenile onset diabetes. Specific effects of this infection on islet cell function were monitored by measuring cellular mRNA content and insulin synthesis. Insulin synthesis was measured by radioactive amino acid incorporation and RIA. Cellular RNA was hybridized with [^{3}H]poly(U) to quantitate total mRNA, with ^{32}P-labeled preproinsulin and somatostatin probes to quantitate these mRNAs, and with a ^{32}P-labeled virus-specific probe to evaluate persistence.

The infected mice (>80%) exhibited hypoglycemia at 72 h postinfection (p.i.) and hyperglycemia at 6 and 8 weeks. Total protein and insulin synthesis decreased in the infected groups compared to the controls. Significant reductions in the levels of cellular mRNAs were also evident during the early and late stages of infection. Infectious virus was recovered from the pancreas and beta cells only at 72 h p.i. However, viral RNA sequences were abundant at 72 h p.i. and in fair amounts later. It appears that coxsackievirus B4 produces a persistent infection in the host, which impairs host cell function to reduce mRNA and insulin synthesis apparently leading to hyperglycemia.

R21.18

MOLECULAR BASIS FOR CLEAVAGE OF THE HEMAGGLUTININ IN INFLUENZA VIRUSES. Y. Kawaoka and R.G. Webster. St. Jude Children's Research Hospital, Memphis, TN, USA

Cleavage of the hemagglutinin (HA) in tissue culture in the absence of trypsin has been correlated with virulence of influenza viruses. To understand the molecular basis for cleavage of the HA molecule, the amino acid sequence of the cleavage site of the HA was compared between virulent and avirulent H5 influenza viruses. Furthermore, the amino acid sequence of the cleavage site was changed in the cloned virulent HA gene by site-specific mutagenesis. The mutagenized HA molecules were expressed in the SV40 system and were examined for cleavage in the absence of trypsin. The importance of basic amino acids at this site for cleavage of the HA will be discussed.

R21.19

COXSACKIEVIRUS B3-INDUCED CELL SURFACE ALTERATION(S) AND MURINE MYOCARDITIS. C. Gauntt, E. Godeny, N. Arizpe, W. Lutton. Department of Microbiology, University of Texas Health Science Center at San Antonio, Texas, U.S.A.

Coxsackievirus B3 (CVB3) induces a cell surface change on neonatal skin or cardiac fibroblasts that is detectable by binding of the lectin Ulex europaeus agglutinin I (UEAI). Cultures of fibroblasts can produce CVB3 for more than a week in absence of cytopathology. Activated natural killer (NK) cells can lyse CVB3-infected neonatal skin fibroblasts within four hours of virus challenge, suggesting NK cell responses to early virus-induced surface alterations. Inhibitors of virus replicaton prevent CVB3-challenged fibroblasts from serving as target cells for NK cells. Fibroblasts challenged with inactivated virus do not serve as target cells. Specific cytotoxic T lymphocytes (CTL) from mice with CVB3-induced myocarditis lyse neonatal skin fibroblasts infected for 72 hr more efficiently than cells infected for 24 or 48 hr. Fibroblasts infected for 72 hr more efficiently bind UEAI than fibroblasts infected for 24, 48 or 96 hr. CVB3 is not known to encode glycoproteins; UEAI-binding proteins are likely of cell origin. Thus, nonlytic CVB3 infection of murine fibroblasts induces cell surface changes detected by UEAI binding and by lysis by activated NK cells and sensitized CTL. Both NK cells and CTL have roles in CVB3-induced myocarditis, possibly through CVB3-induced surface changes on cardiac fibroblasts.

R21.20

HUMAN IMMUNODEFICIENCY VIRUS ISOLATES DIFFER IN REPLICATION POTENTIAL IN VITRO, Eva Maria Fenyö, Francesca Chiodi, Jan Albert and Birgitta Åsjö, Dept of Virology, Karolinska Institutet, Stockholm, Sweden.

Human immunodeficiency virus (HIV) has been isolated from 33 HIV antibody positive individuals with different clinical manifestations of infection. Peripheral blood mononuclear cells (PBMC) from AIDS or pre-AIDS patients yielded virus rapidly as detected by high reverse transcriptase (RT) activity in culture fluids. These viruses were able to establish a persistent infection in several T4 antigen positive tumor cell lines (CEM, H9 and U937 clone 2) and were designated rapid/high. PBMC cultures from individuals with mild or no symptoms yielded virus more slowly and the RT activity was low. Cocultivation of PBMC yielding such slow/low viruses with the T4 positive tumor cell lines showed no or only transient virus replication, as a rule. Cell free transmission of viruses to PBMC from normal donors and to cell lines showed that viruses classified as rapid/high are readily transmitted whereas viruses of the slow/low type replicate poorly, if at all. In fact, slow/low viruses could be divided into 4 groups on the basis of their transmissibility and growth properties. Viruses in group 3 and 4 could efficiently replicate in the Jurkat/tat$_{III}$ cell line allowing radioimmunoprecipitation and restriction enzyme analysis.

R21.21

EFFECT OF DENGUE VIRUS INFECTION ON MACROPHAGE FUNCTIONS
U.C. Chaturvedi, R. Nagar, N. Rizvi, A. Mathur, Department of Microbiology, K.G. Medical College, Lucknow, India.

Dengue virus (DV) infection in mice adversely affects the macrophages, killing nearly two thirds of them and depressing the functions of the remaining ones. The depressed functions are phagocytosis, migration on glass surface, capacity to bind and the capacity to present homologous or heterologous antigens by the macrophages. DV replicates in both I-A positive as well as I-A negative macrophages. The adverse effects on macrophages do not appear to be due to the replication of the virus alone, but are mediated by the production of soluble factors. DV induces a subpopulation of T lymphocytes to produce a cytotoxic factor (CF) which induces I-A positive macrophages to produce a soluble cytotoxin(CF_2). Both these factors kill normal lymphoid cells and macrophages in vivo and in vitro; the cells which survive loose different functions, thus suppressing immune response and immunopathological effects.

R21.22

SECONDARY IMMUNE RESPONSE TO REACTIVATED JAPANESE ENCEPHALIS VIRUS IN LATENTLY INFECTED MICE. Asha Mathur, R. Kulshreshtha & U.C. Chaturvedi, Department of Microbiology, K.G. Medical College, Lucknow, India.

Secondary immune response to Japanese encephalitis virus (JEV) has been demonstrated following reactivation of the virus in latently infected mice. The development of cell mediated immune responses were accelerated but short lived as compared with the primary response. Passively transferred serum and splenic T cells obtained upto 1 week after reactivation of the virus provided protection against a challenge infection with JEV. Antigen-specific suppressor T cells (Ts) for DTH and humoral response develop in JEV-infected mice. During the virus reactivation secondary Ts cells are also produced at a faster rate. It is concluded that specific memory cells for the immune response persist in mice having latent JEV infection.

R21.23

HIGH FREQUENCY OF REINFECTION OF SEROPOSITIVE RENAL ALLOGRAFT RECIPIENTS WITH CMV FROM DONOR KIDNEY AND ITS ASSOCIATION WITH SYMPTOMATIC ILLNESS.
Jane E. Grundy[1], S.F. Lui[1], M. Super[1], P. Sweny[2], & P.D. Griffiths[1], Departments of Virology[1] and Nephrology & Transplantation[2], Royal Free Hospital, London NW3, England.

Cytomegalovirus (CMV) infection is a major cause of morbidity and mortality in renal allograft recipients. The source of CMV infection in seronegative recipients has been shown to be the donor kidney. In seropositive recipients the source of virus could either be reactivation of the recipient's own virus or reinfection with donor virus. We have used restriction enzyme analysis of the viral DNA to establish the origin of the virus excreted in recipient pairs with a single kidney donor. In each pair studied the same strain of CMV was excreted by both recipients, indicating that the source of the virus in the seropositive recipient was reinfection from the donor kidney. Furthermore, from the difference in the excretion rate of CMV in seropositive recipients with seronegative donors (reactivation: 69%) and those with seropositive donors (reinfection-plus-reactivation: 69%), we estimate that the incidence of reinfection is likely to be around 42%. Thus reinfection may be almost twice as common as reactivation in seropositive recipients. In addition symptomatic illness occurred only in recipients with proven reinfection (by DNA typing of the virus strain), or in recipients with a seropositive donor, and not in those with proven reactivations.

R21.24

ANALYSES OF MONONUCLEAR CELL-ASSOCIATED VIREMIA IN NON-IMMUNOCOMPROMISED CHILDREN WITH VARICELLA. N. Itakura, S. Hirose, S. Suga, Y. Asano. Departments of Pediatrics, Kariya Sogo Hospital and Fujita Gakuen Health University School of Medicine, Aichi, Japan.

For quantitative and qualitative analyses of viremia in varicella in relation to clinical signs of the disease, peripheral blood mononuclear cells (PBMCs) from children after close exposure to natural varicella-zoster virus (VZV) were; 1) serially diluted, 2) fractionated to several cell subpopulations, and then applied for VZV isolation using the human embryonic lung cell system. VZV was isolated from PBMCs in 15 (83%) of 18 children 1 to 3 days before onset of the disease. Estimated number of VZV-infected PBMCs in blood determined by serial dilution of cells was 1.2 to 83.3 cells per 10^7 PBMCs, which was correlated well with the number of skin vesicles but not with a febrile episode. After fractionation of PBMCs, VZV was isolated from non-adherent cell in 10 (91%) of 11, adherent cells in 1 (7%) of 15, T-cells in 9 (60%) of 15, and B-cells in 6 (40%) of 15. One child with the most severe signs of disease had the virus in all of the cell subpopulations. The procedures for cell fractionation increased the detection of VZV viremia.

R21.25

THE PRODUCTIVE DNA REPLICATION OF EPSTEIN-BARR VIRUS. Pamela J. Siegel Randolph. Department of Biology, Boston University, Boston, Massachusetts 02215 U.S.A.

We have studied viral DNA production of the human B-lymphotrophic Epstein-Barr virus for several years. From studies to date, we have concluded that linear viral DNA is produced via bidirectional replication of a relaxed circular intermediate. The precursor to linear viral DNA was shown to be a 65S intermediate in a study (see Siegel et al. J. Virol. 38, 880.) using physical chemical analysis. More recent data from Hind III restriction digests showed the linear DNA left end (A) to be joined to the right end (D_1 or D_{1het}) in that precursor, conclusively demonstrating it to be a relaxed circular species. In addition, this conclusion was consistent with some of the earliest work by several groups on EBV DNA replication, which also demonstrated that the EBV genome existed as a plasmid within the cell. Non-toxic levels of the intercalating dye ethidium bromide reduced virus production in the P3-HR1 producer cell line by fifty percent; biologically, the conclusion has proven correct, as well.

Enzymes which help replicate and unwind circular DNA will be important targets for anti-EBV (and CMV) agents. Studies with topoisomerase inhibitors are currently underway; hopefully, immune suppressed patients as well as those suffering from acute and chronic infections would benefit.

Tentatively, two lytic origins exist and differ from the mapped plasmid origin for DNA replication.

R21.26

ENHANCEMENT OF NATURAL KILLER CELL ACTIVITY BY HUMAN CYTOMEGALOVIRUS PERSISTENTLY INFECTED CULTURE.
T. Murayama, Xu Bin, K. Shimokawa, T. Furukawa.
Department of Microbiology, Kanazawa Medical University, Uchinada, Ishikawa, JAPAN.

We reported that infection of cells derived from an human osteogenic sarcoma (HOS) with human cytomegalovirus (HCMV) resulted in persistent infection (E155 cells). The present work was designed to examine the effect of persistent infection on natural killer (NK) cell activity in peripheral blood lymphocytes (PBL) obtained from HCMV-seropositive and seronegative individuals against K562 cells. NK activity of normal PBL demonstrated about 30 % in both HCMV-seropositive and seronegative subjects, whereas increased to about 60 % by cocultured PBL with E155 cells for 5 days. The highest enhancement of NK activity was observed by the cocultured PBL with E155 cells at the ratio of 10:1. This enhanced effect was observed neither by PBL cocultured with HOS cells nor cell free-HCMV. Increased lysis by cocultured PBL with E155 cells were observed not only against K562, but also against HOS and E155 cells. Normal PBL displayed the lytic activity of 7 % for HOS cells and 8 % for E155 cells, while lysis by cocultured PBL with E155 cells was 28 % and 40 %, respectively. Monoclonal antibody-complement depletion studies indicated that the K562 and E155 cells were lysed by OKT3 negative and Leu 7 positive PBL. Thus, NK activity in PBL can be stimulated by cocultvation with E155 cells.

R21.27

NUCLEOTIDE SEQUENCING OF THE GLYCOPROTEIN GENES OF CANINE DISTEMPER VIRUS. D.K. Clarke, M. Nosita, & B.K. Rima, and S. Evans & T. Barrett, Dept. of Biochemistry, Queen's University of Belfast, N. Ireland and Animal Virus Research Institute, Pirbright, England.

We have prepared a cDNA clone bank of the Onderstepoort strain of canine distemper virus (CDV). Sequence determination has been primarily focussed on the genes encoding the F and 'H' glycoproteins. The sequence of the F protein is compared to that of measles (MV) virus. The F gene of CDV is 2149 nucleotides in length which is 122 nucleotides shorter than that of MV. Whereas the translation of MV F mRNA starts at an AUG codon at position 583 in MV encoding a protein of 550 amino acid residues (Richardson et al. Virol.155, 508), in CDV there is an ORF starting at position 86 in the mRNA which can be translated to a protein of 656 residues. This is larger than expected from the proteins sized on SDS-PAGE. The F1 portion of the proteins are well conserved between CDV and MV with an overall homology of 64%. Divergence is found in the putative membrane anchor, in the cytoplasmic domain and in the F2 part of the protein. Glycosylation sites are only found in F2. The sequence of the H protein will also be compared to that of the known sequences of a number of MV strains. So far a wide divergence between CDV and MV sequences has been observed. The intergenic regions between the N/P and P/M genes have also been sequenced.

R21.28

THE ROLE OF LEUKOKINES IN JAAGSIEKTE. M.S. Myer, D.W. Verwoerd and H.M. Garnett*, Department of Molecular Biology, VRI, Onderstepoort, 0110, South Africa; *Department of Biology, University of Wollongong, Northfield Avenue, Wollongong, NSW, Australia, 2500.

The characteristic increase of alveolar macrophages in the lungs of jaagsiekte sheep is initiated by a tumour cell-derived chemotactic factor that is protein in nature with a molecular mass in the region of 13 kilodaltons (kDa). This polypeptide was found to stimulate a mixed culture of alveolar macrophages and other free cells in lung washes to produce two polypeptides, in the region of 13-25 kDa and below 10 kDa, which stimulate and inhibit DNA synthesis in tumour cells, respectively.

A model is presented of a tumour cell-derived macrophage chemotactic factor stimulating the overall growth of tumour cells via an interaction between alveolar macrophages and the other cells present in a lung wash.

R21.29

RETROVIRUSES AND DISEASE: ANALYSIS OF CIRCULATING IMMUNE COMPLEXES FROM PATIENTS WITH ANKYLOSING SPONDYLITIS.
E. Rødahl, O.-J. Iversen. Dept. of Microbiology, University Hospital, N-7000 Trondheim, Norway.

In animals suffering from chronic inflammatory disorders induced by retroviruses, immune reactions against the viral envelope proteins are observed. In some cases, the envelope proteins have been detected in circulating immune complexes (CIC).

Recently, we have isolated a retrovirus-like particle from a patient with psoriasis. The particle was composed of two envelope proteins with Mr of 70 and 15 kD, and three internal proteins of 27, 15 and 12 kD. A possible role of the major internal protein, p27, in the pathogenesis of psoriasis has been described previously. In this study, we have paid attention to ankylosing spondylitis (AS), a disorder closely related to psoriatic arthritis.

Antiserum against the 70 kD envelope glycoprotein of the psoriasis associated retrovirus-like particle (gp70), was obtained by immunising rabbits with purified gp70. CIC were isolated from AS patients by isopycnic ultracentrifugation in sucrose gradients. Immunoblot analyses revealed the presence of a 70 kD and a 40-45 kD antigen cross-reacting with anti-gp70 antiserum in 4 out of the 7 CIC samples studied, indicating that a retrovirus-related antigen may participate in immune complex formation in AS.

R21.30

REPLICATION OF CYTOMEGALOVIRUS IN HUMAN THYMIC EPITHELIAL CELLS. K. Numazaki, H. Goldman, I. Wong, M.A. Wainberg. Montreal Children's Hospital Research Institute and Jewish General Hospital, Montreal, Quebec, Canada.

Cytomegalovirus (CMV) has often been cited as a cause of immune suppression in children, yet little is known of the mechanisms through which this virus might affect immune function. We have succeeded in using CMV to productively infect cultures of human thymic epithelial (TE) cells. In the case of TE cells which were obtained from aborted fetuses, cytopathic effects (CPE) were apparent by 48 hr after infection; CMV early antigen (EA) and late antigen (LA) were detected by immunofluorescence after 8 days, and infectious progeny virus was recovered from the culture medium by day 12. When TE cells, which were derived from cases of pediatric cardiovascular surgery were employed, no CPE was observed, yet cells became positive for EA and LA after 12 days, and infectious progeny virus was harvested after 17 days of culture. In view of the fact that TE cells are known to play an important role in T cell maturation and differentiation, these findings suggest that CMV infection of the human thymus might potentially play a role in virus-mediated immune suppression. Supported by the Medical Research Council of Canada.

R21.31

NEUTRALIZATION OF THE INTERFERON INDUCED PROTEIN MX IN MOUSE AND RAT CELLS BY MICROINJECTION OF MONOCLONAL ANTIBODIES. H. Arnheiter, E. Meier*, G. Weitz, L. Richter-Mann, and O. Haller*. Lab. of Molecular Genetics, NINCDS, NIH, Bethesda, Maryland, USA, and *Inst. For Immunology and Virology, University of Zuerich, Zuerich, Switzerland.

Interferon (IFN) induces in cells a number of proteins, some of which are involved in the establishment of protection against viral infections. One of them, the 70 kD Mx protein originally detected in IFN alpha/beta treated cells from influenza virus resistant mice, is both necessary and sufficient for protection against influenza viruses in mouse cells (Staeheli et al., Cell 44, 147-158, 1986). In rat cells, at least three Mx related proteins are translated from messages with extensive homology to the mouse Mx mRNA, and in human cells, there is just one Mx related protein. While the mouse and rat proteins accumulate in the nucleus, the human protein is strictly cytoplasmic. To test the role of these Mx related proteins, we microinjected monoclonal anti-Mx antibodies into cultured mouse, rat and human fibroblasts, treated the cells with the respective interferons, and infected them with either influenza or vesicular stomatitis virus. Using a single cell fluorescent assay, we found that monoclonal antibody 2C12 neutralizes protection against influenza virus in a dose dependent manner in both mouse and rat cells without affecting the protection against VSV. However, microinjection had no effect on protection against influenza virus nor VSV in human cells. Further studies are under way to test the function of the human Mx protein either purified from induced human cells, or expressed by recombinant DNA techniques.

R21.32

MEASLES VIRUS RNA DETECTED IN NON-CNS TISSUES IN SSPE. H.R. Brown,[1] N.L. Geller,[1] J. Dymecki,[2] H.H. Wisniewski.[1] [1]NYS Office of Mental Retardation & Developmental Disabilities, Institute for Basic Research, Staten Island, N.Y., U.S.A. and [2]Psychoneurological Institute, Warsaw, Poland.

Subacute sclerosing panencephalitis (SSPE), a slowly progressing fatal disease of the CNS of children, is caused by persistent infection with measles virus (MV). Acute measles generally precedes signs of SSPE by several years, but it is not known where MV persists while disease is inapparent. High levels of serum antibodies to MV are characteristic and elevated serum IgM levels during different clinical stages have been reported, suggesting continuous antigenic stimulus. However, involvement of organs outside the CNS has rarely been documented. To search for such peripheral reservoirs of MV, we have done in situ hybridization to detect MV RNA and immunostaining to locate MV antigens in tissue sections of organs from post mortem cases of SSPE. Immunostaining failed to detect MV antigens in any of 73 blocks from 5 SSPE cases. In situ hybridization with a cloned cDNA probe for MV nucleocapsid protein (NP) (gift of S. Rozenblatt, Weizmann Institute) indicated presence of MV RNA in 7 of 21 blocks from one case tested thus far. Positive tissues included tonsil, adrenal, pancreas, lung, and pituitary. Our findings indicate that (1) in situ hybridization can detect MV infected cells which might otherwise have been missed, and (2) that MV is indeed present in tissue outside the central nervous system in SSPE.

R21.33

IDENTIFICATION OF A HUMAN KIDNEY CELL TYPE PRODUCTIVE FOR HUMAN CYTOMEGALOVIRUS (HCMV) INFECTION IN VITRO. M.H. Heieren, H.H. Balfour, Jr., Department of Laboratory Medicine/Pathology, University of Minnesota, Minneapolis, MN, USA.

Highly productive replication of HCMV in vitro is restricted to human fibroblasts. Infectious center assays of primary mixed kidney cortex cell cultures infected with HCMV fail to support significant virus replication as compared with skin fibroblasts (SF) using either laboratory or clinical HCMV strains (0.2% vs. 65% respectively). However, HCMV DNA replication was readily detected by dot blot hybridization of purified kidney cell nucleic acid with a ^{32}P nick-translated HCMV probe. Biotin/streptavidin in situ hybridization and HCMV specific monoclonal immunofluorescence showed that virus expression was concentrated in a morphologically distinct cell type identified as kidney mesangium.

Virus yield assays of purified mesangium produced 10^7 pfu/ml as compared with SF and mixed kidney cortex cultures (10^6 pfu/ml and 10^3 pfu/ml respectively). Infected mesangium produced HCMV specific IE proteins by SDS-PAGE, initiated HCMV DNA replication by dot blot hybridization, and expressed HCMV late antigen by monoclonal immunofluorescence. We conclude that human kidney mesangial cells are highly productive for HCMV replication in vitro. These findings suggest that HCMV infected mesangium contributes to CMV-induced renal disease in vivo.

R21.34

IMMUNOSUPPRESSION IN RABBITS INFECTED WITH BOVINE LEUKEMIA VIRUS. C. Wyatt, C. Buck, R. Reeves, J. Evermann, A. Rousseau, J. Magnuson, N. Magnuson. Departments of Micro, Genet./Cell Biology, Biochemistry, Vet./Micro. Path. Washington State Univ., Pullman, Wa., USA

Bovine leukemia virus (BLV), an exogenous B lymphocyte lymphoma and leukemia-inducing retrovirus of cattle, belongs to the same family as, and shares homology with, the etiologic agent of human adult T-cell leukemia (HTLV-1). Our studies show that 1) BLV can infect young adult rabbits and that 2) infected rabbits have depressed *in vitro* T-lymphocyte responses. Infected rabbits have serum antibody to BLV that persists for months without re-inoculation. Cultured lymphocytes from infected rabbits induce syncytia in indicator cells and antiserum to BLV added to these cultures reduces syncytia induction by >90%. Reverse transcriptase activity can be detected in supernatants of lymphocytes (1.2-4.1 pm product/culture/hour) from these rabbits. *In vitro* T lymphocyte responses of rabbits to phytlectins show significant ($p=0.05$) and prolonged depression (1-8 months). We believe that BLV infection of rabbits is an excellent model system for the study of BLV and related viruses.

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R21.35

CHRONIC INFECTION OF MICE WITH PICHINDE VIRUS. K. E. Wright, M. J. Buchmeier. Department of Immunology, Research Institute of Scripps Clinic, La Jolla, CA 92037, USA.

There are many animal models of viral infections where infectious virus persists for life and may or may not cause disease. There are few models of chronic infection where infectious virus is cleared, but viral antigen persists at the same time that disease progresses. It has been reported that intracranial infection of neonatal mice with the arenavirus Pichinde (PV) is fatal. We have found however that infection of neonates is not always fatal. Some strains of mice, as typified by BALB/c, survive and become chronically infected. Infectious virus persists in the liver and spleen for 60 days, but in a proportion of mice persists in the brain and kidney for up to 9 months. Viral antigen can also be detected in the spleen, kidney and brain at this time. There is no pathology in any of the organs except the kidney, which shows evidence of glomerular disease. Possible mechanisms underlying the persistence of PV in BALB/c and other strains of mice are being examined.

R21.36

PROTECTIVE MECHANISMS AGAINST EMC-D VIRUS INDUCED DIABETES BY CORYNEBACTERIUM PARVUM. S. Nagafuchi, E. Kounoue, M. Nakamura, R. Iwakiri, S. Nakano, T. Koga, M. Nakayama, Y. Niho, R. Takaki*, The First Department of Medicine, Faculty of Medicine, Kyushu University, Fukuoka, and the Department of Medicine 1*, Medical College of Oita, Oita, Japan

Corynebacterium parvum (CP) protected against development of EMC-D virus-induced diabetes mellitus (DM) in mice, when administered 5 to 14 days previously. This pretreatment with CP significantly inhibited growth of the virus in various organs, including the pancreas, at an early stage of the infection, thus preserving the insulin contents of pancreas and inhibiting the development of DM. Neutralizing antibody appeared on the 4th day and a plateau was reached at 10 to 14 days after infection, in both CP treated and non-treated mice. No significant difference between the groups was observed. Anti-EMC-D virus antibodies raised in rabbits inhibited the development of DM, when transferred to mice within 36 hrs of infection with the virus, thus the naturally occurring antibody apparantly did not inhibit development of DM. Production of interferon was low in both control and CP pretreated mice following the virus infection, thereby suggesting that interferon may not be involved in the CP mediated protection. In contrast, peritoneal macrophages derived from mice pretreated with CP significantly inhibited the growth of the virus in vitro, thereby indicating that direct macrophage activation plays a central role in CP mediated DM inhibition.

R21.37

RABBIT MODEL OF ROTAVIRUS INFECTION. M.E. Conner, M.K. Estes, D.Y. Graham. Departments of Virology and Epidemiology and of Medicine, Baylor College of Medicine, Houston, Texas USA.

We developed a small animal model to examine active immune responses and to evaluate candidate vaccines. Seronegative neonatal to 16 week old NZW rabbits were inoculated orally with cultivatible rabbit rotaviruses C11 and Ala and simian rotavirus SA11, and the course of the disease was evaluated by clinical findings, virus isolation (plaque assay and ELISA), and examination of tissues (histopathology and immunocytochemistry). Virus excretion in intestinal contents was detected from 6 hours to 7 days postinoculation. RNA electropherotypes of inocula and reisolated viruses were the same in all samples tested. In a challenge experiment 39 and 58 day old rabbits were inoculated and then challenged 5 weeks later with Ala virus. Virus was excreted in feces from 2 to 9 days postinoculation but was not excreted following challenge. Rabbits seroconverted following inoculation. An advantage of this model is that rabbits remain susceptible to infection to at least 16 weeks of age (upper limit examined). Therefore, the rabbit provides an ideal model to investigate the active immune response to rotavirus infections.

R21.38

PERSISTENT CO-INFECTION OF T-LYMPHOCYTES WITH HTLV-II AND HIV AND THE ROLE OF SYNCYTIUM FORMATION IN HIV-INDUCED CYTOPATHIC EFFECT. David C. Montefiori, W. Edward Robinson and William M. Mitchell, Vanderbilt University, School of Medicine, Nashville, Tennessee, USA.

We previously demonstrated a high permissiveness of HTLV-II-transformed T lymphocytes (C3) to human immunodeficiency virus (HIV) infection in vitro, and that this infection results in the lysis of cells (D.C. Montefiori and W.M. Mitchell, Virology, 155, 726-731, 1986). We now show that a small percentage of HIV infected C3 cells resist cell lysis, grow continuously in culture and express antigens of both viruses. High levels of reverse transcriptase activity found in the culture fluid of these co-infected cells was associated with the presence of fully infectious HIV and an absence of detectable infectious HTLV-II. Virus production in C3 cells co-infected with HIV isolate HTLV-III was approximately 3-fold greater than in C3 cells co-infected with the HIV isolate LAV, a result which suggests that HIV genomic diversity may give rise to differences in replicative capacities. Lysis resistance was found to be a cellular-determined function in that HIV produced in cultures of C3/HTLV-III cells retained the capacity to elicit a lytic response upon repeated infection. Small syncytia were rarely observed in cultures of C3 and non-lytic C3/HIV cells whereas large syncytia were in abundance during the lytic phase of co-infections, a result which supports a role for syncytium formation in the mechanism of HIV-induced cytopathic effects.

R21.39

CELLULAR PERMISSIVENESS TO MURINE HEPATITIS VIRUS INFECTION. C. Kooi, M. Daya, C. Alderson, R. Anderson. Department of Microbiology and Infectious Diseases, University of Calgary, Calgary, Alberta, Canada, T2N 4N1.

Mouse hepatitis virus (MHV) establishes a lytic infection in the L-2 subline of mouse fibroblasts but a persistent infection in the LM-K subline. MHV infection of LM-K cells occurs at a level of approximately 1/500 that of L-2 cells. The basis for this difference involves an early event in MHV replication. Assays of virus adsorption, using radiolabeled MHV virions showed only a 6-fold higher degree of binding of MHV to L-2 as compared to LM-K cells. Binding studies with additional cell lines showed a lack of correlation between MHV binding and degree of permissiveness to infection. Kinetics of MHV internalization in L-2 and LM-K cells were similar; however total numbers of internalized infectious virions were 25-fold higher in the case of L-2 cells. This result was supported by preliminary studies using dot blot hybridization of internalized inoculum RNA. We therefore conclude that cell differences in virus internalization play a major role in determining MHV susceptibility and the subsequent outcome of MHV infection.

R21.40

ABSENCE OF EVIDENCE OF TRANSMISSION OF HIV, HTLV-I, STLV-III, AND SRV-I TO LABORATORY AND ANIMAL CARE PERSONNEL. D. Madden*, W. London*, N. Tzan*, D. Fuccillo**, J. Sever*. *NINCDS, NIH, Bethesda, MD, **Microbiological Associates, Bethesda, MD.

Concern for the safety of laboratory and animal care workers exposed to human (HIV, HTLV-I) and non-human primate retroviruses (STLV-III, SRV-I) has become an important consideration in working with laboratory specimens and infected animals. We have been actively involved in AIDS and SAIDS research in man and non-human primates since 1982. We have maintained a P2 facility and have included a health evaluation program for our employees which include semi-annual collection and storage of blood samples and routine testing for infectious agents which are being studied in the laboratory. We have studied serial specimens from 10 laboratory and 6 animal care individuals working with these agents in the man and animals obtained since 1980 or time of employment until January 1987. No known needle exposure or prolonged illness has occurred. All serum samples were tested by indirectfluorescent antibody tests using control positive and negative antigens and serum against HIV, HTLV-I, STLV-III and SRV-I. All seral serum specimens were antibody negative for all four viruses. Thus, in our P2 facility there has been no evidence of transmission of these viruses to laboratory or animal care workers.

R21.41

REOVIRUS CELL ATTACHMENT PROTEIN σ1 TRIGGERS THE INDUCTION OF AUTOANTIBODIES IN MICE. C. Pak, M.C. Yeung, J.-W. Yoon, and P.W.K. Lee. Department of Microbiology and Infectious Diseases, University of Calgary, Calgary, Canada.

It was previously reported that reovirus type I triggers a polyendocrine disease and induces autoantibodies against cytoplasmic antigens in the islets of Langerhans, anterior pituitary and gastric mucosa. The reovirus S1 gene which encodes the σ1 protein was suggested to be responsible for the induction of autoantibodies to growth hormone. To determine directly whether σ1 protein by itself can induce autoantibodies to islets, stomach and anterior pituitary, 3 week old male SJL/J mice were immunized four times with purified σ1 protein at weekly intervals. Sera were collected and assayed for the presence of autoantibodies against normal SJL/J mouse tissues, using the indirect immunofluorescent technique. Approximately 47 to 53% of mice immunized with reovirus type 1 σ1 produced autoantibodies against pancreatic islets and gastric mucosa, while only 5 to 10% of mice immunized with the reovirus type 3 protein had such autoantibodies. In addition, 6% of mice immunized with reovirus type 1 σ1 protein also produced autoantibodies against the anterior pituitary gland. None of the control mice had such autoantibodies. It is concluded that (1) protein σ1 alone can induce autoantibodies against islets, gastric mucosa and anterior pituitary, and (2) there is a viral serotype specificity in the induction of autoantibody.

R21.42

EFFECTS OF NATURAL KILLER (NK) AND LYMPHOKINE ACTIVATED KILLER (LAK) CELLS ON MURINE VIRUS INFECTIONS. R.M. Welsh, R.J. Natuk, K.W. McIntyre, H. Yang, and J.F. Bukowski. Dept. of Pathology, University of Massachusetts Medical School, Worcester, MA 01605, USA.

Infections of mice with murine cytomegalovirus (MCMV) or lymphocytic choriomeningitis virus (LCMV) resulted in activation and accumulation of NK cells in virus-infected tissue. Peritoneal fluid from virus-infected mice, as well as both human recombinant interleukin-2 (IL-2) and purified mouse beta interferon (IFN), were chemotactic for NK cells in in vitro Boyden chamber assays. Depletion of NK cell activity in vivo by antibody to asialo GM1 rendered mice more sensitive to MCMV but not to LCMV. Adoptive transfer of non-immune adult leukocytes into NK-deficient baby mice protected mice against MCMV but not LCMV, and the protection correlated with the presence of NK cells. LAK cells were generated by cultivating mouse spleen cells for 5 days in 100 U/ml IL-2. High levels of LAK cell cytotoxicity were mostly mediated by subpopulations bearing the NK cell phenotype, and these LAK cells responded chemotactically to purified IFN-beta. Transfer of as few as $5x10^5$ LAK cells into baby mice protected them against MCMV but not LCMV. Thus, infection by an apparent NK-sensitive virus (MCMV) but not by an NK-resistant virus (LCMV) can be regulated by LAK cells, whose cytotoxicity in vitro appears to be mediated by an activated NK cell subpopulation.

R21.43

A DECREASED VIRUS-SPECIFIC CYTOTOXIC T CELL RESPONSE FOLLOWING NEONATAL INFECTION WITH NEUROTROPIC Cas-Br-M MuLV. D.S. Robbins, P.M. Hoffman. University of Maryland at Baltimore and VA Medical Center, Baltimore MD, 21201, U.S.A.

Maturation of T cell immunity is a major host factor in determining the age-acquired resistance to Cas-Br-M MuLV induced spongiform neurodegeneration in susceptible inbred NFS/N mice. We have examined the role of cytotoxic T lymphocytes (CTL) as the possible effector cells in this immune mediated resistance to retrovirus induced neurodegeneration. Using an in vitro Cas-Br-M MuLV specific, MHC restricted cytotoxic T cell assay with a Cas-Br-M MuLV infected pre-B lymphoma cell line (NS467) as a target cell, we measured cytotoxicity by ^{51}Cr release after a 5 day in vitro restimulation of spleen cells. Cytotoxicity was detected 1 week post-infection and remained at high levels (60-70% killing) for more than 20 weeks in immune mice that never developed neurodegenerative disease. In contrast, NFS/N mice infected at birth developed neurologic disease but failed to demonstrate a virus specific CTL response until 3 weeks post-infection. Cytotoxicity greater than 25% killing did not occur during the course of neurologic disease, even though these mice mounted a normal allogeneic CTL response. We conclude that development of an MuLV specific CTL response may play an important role in resistance to Cas-Br-M MuLV neurodegenerative disease.

R21.44

CYTOTOXIC ACTIVITY OF PULMONARY CELLS IN CYTOMEGALOVIRUS (CMV) INTERSTITIAL PNEUMONIA (IP) FOLLOWING MARROW TRANSPLANT. Raleigh A. Bowden, Sherrie Dobbs, Joel D. Meyers. Fred Hutchinson Cancer Research Center, Seattle, WA, USA

Cytotoxicity by bronchoalveolar lavage cells obtained from 14 IP patients was evaluated as a possible explanation for the progression of CMVIP after marrow transplant. 7 patients had fatal CMVIP, 3 survived CMVIP and 4 had non-CMVIP. Ficoll-Hypaque separated cells (F-H) from patients with CMVIP, tested for lysis of CMV-infected fibroblast targets in an 18 hour 51Cr release assay, had a mean lysis of 37% compared to 14% by cells from patients without CMVIP. Plastic adherent cells (Ad) and nylon wool non-adherent cells (NA) showed a mean lysis of CMV targets of 9% by Ad compared to 26% lysis by NA. However, Ad from 2 of 3 patients with fatal CMVIP also had 17% lysis of CMV targets. Lysis of uninfected targets by F-H was 21% in patients with fatal CMVIP compared to non-fatal CMVIP (5%) and non-CMVIP (7%). Lysis by F-H cells was higher than by Ad or NA tested separately, suggesting soluble factors may enhance nonspecific cytotoxicity. In summary, cytotoxicity against CMV targets by F-H cells from patients with CMVIP was greater than by cells from patients with non-CMVIP. Cytotoxicity against CMV targets was due primarily to NA, although Ad also contributed to cytotoxicity in patients with fatal outcomes. Lysis of uninfected targets by F-H cells in patients with fatal outcomes suggests that non-specific cytotoxicity, perhaps mediated by Ad, may also be involved in the pathogenesis of CMVIP after transplant.

R21.45

HUMORAL IMMUNE RESPONSE TO EXPERIMENTAL SIV/DELTA INFECTION IN RHESUS MACAQUES. Michael Murphey-Corb, Jing-yu Zhang, and Louis N. Martin, Delta Regional Primate Research Center, Tulane University, Covington, LA, U.S.A.

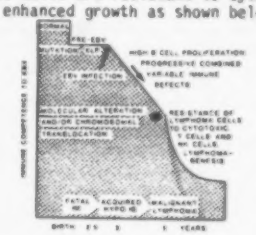
We have examined SIV/Delta-related pathogenesis of in vitro propagated virus isolated from several rhesus with SAIDS and one asymptomatic mangabey. Several isolates of both rhesus and mangabey origin are pathogenic; one isolate is singularly neurovirulent. One isolate, after long-term propagation in vitro, is no longer virulent.

Western blot analysis of serial sera obtained from animals inoculated with these isolates has revealed the following observations. Antibody to both gag and env-related viral determinants was detected 1-4 weeks post infection regardless of the inoculum used which persisted in clinically healthy animals but which declined in a predictive fashion before an animals' death with opportunistic infection. This decline was independent of elevated serum immunoglobulin and B cell hyperplasia observed in some animals. One animal developed lymphoma; persistent antibody titers were noted throughout the course of the disease. In contrast, animals with retroviral encephalitis produced little SIV-specific antibody at any time during the course of the disease; this lack of antibody production correlated with an early and severe decline in serum immunoglobulin. This observation is particularly intriguing since SAIDS neuropathy may be strain specific.

R21.46

MECHANISMS OF EPSTEIN-BARR VIRUS-INDUCED LYMPHOPROLIFERATIVE DISEASES IN THE X-LINKED LYMPHOPROLIFERATIVE SYNDROME (XLP). D. Purtilo, H. Grierson, Dept. Pathology/Microbiology, Univ. Nebraska, Omaha, NE 68105.

About 60% of patients develop fatal infectious mononucleosis (IM), 25% hypogammaglobulinemia with activation of suppressor T cells, and 20% malignant B cell lymphoma. Defective regulation of cytotoxic T cells responding to EBV-infected B cells destroy hepatocytes and marrow cells. Multiple immune defects prevail in survivors. Southern blot analysis with EBV, J_H, and c-myc probes reveal oligo- and monoclonal lesions concurrently with c-myc rearrangement in tissues from fatal IM. Lymphoma likely results from immunoglobulin gene and c-myc alterations as in Burkitt's lymphoma. These cells are resistant to cytotoxic and NK cells and show enhanced growth as shown below. Supported by PHS CA30196.



R21.47

GENETIC AND BIOCHEMICAL STUDIES OF REOVIRUS INTESTINAL GROWTH. D. Bodkin, M. Nibert and B. Fields. Department of Microbiology and Molecular Genetics. Harvard Medical School, Boston, MA USA

Reovirus serotype 1 Lang grows in intestinal tissue of neonatal mice while reovirus serotype 3 Dearing does not grow in intestinal tissue. Previous studies had shown that the difference was a property of the M2 gene (Rubin and Fields, 1980). However, recent studies which raised the question of whether the L2 gene might be involved in viral growth in intestinal tissue (Kerouac and Fields, 1986) caused us to reexamine the growth of the virus in the intestine using reassortants generated from non-mutagenized virus stocks. In these studies the ability of the virus to grow in intestinal tissue appeared to be a property of the L2 and S1 genes.

Biochemical aspects of virus intestinal growth in neonatal mice have been examined using radiolabeled virus as well as biochemical markers for the proteolytic digestion of the virus. It appears that the time course of digestion, as well as the particular proteins digested is roughly equivalent to that seen in in vitro experiments.

R21.48

CLEAVAGE PRODUCTS OF THE REOVIRUS μ1 PROTEIN. M. Nibert, A. Jayasuriya, B. Fields. Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, MA USA

The reovirus μ1C protein is a major component of the virion outer capsid. μ1C is formed during viral assembly by cleavage of the translation product of the M2 gene, μ1. μ1C is additionally cleaved at early times of infection to form the stable product δ. This latter cleavage is thought to be an essential step in the processes of reovirus entry into cells and activation of the viral transcriptase.

In order to study the role of μ1C in reovirus infection, we have sequenced the M2 gene of reovirus T3 Dearing and have identified the locations of the assembly- and entry-related cleavages at either end of the μ1 protein. We have demonstrated that the carboxy-terminal polypeptide fragment generated from the cleavage of μ1C to δ is resistant to further proteolytic digestion and remains virion-associated. We have also found that the μ1C protein in purified virions exists as a disulfide-bonded dimer and that the cysteine mediating dimerization is located in the region of the carboxy-terminal fragment of μ1C.

R21.49

STRUCTURE AND FUNCTION OF THE REOVIRUS MAJOR OUTER CAPSID PROTEIN, σ3. L. Schiff-Maker, M. Nibert, M.S. Co, E. Brown, and B. Fields. Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, MA USA

The reovirus S4 gene product, σ3, comprises the majority of the viral outer capsid. σ3 has been shown to possess dsRNA binding activity and has been proposed to play a role in the regulation of translation in infected cells. Genetic studies have suggested that the S4 gene product is involved in the inhibition of host cell RNA and protein synthesis.

In order to begin to identify the molecular basis for those properties of σ3 which influence the virus-host cell interaction, we have localized an RNA-binding domain on σ3 using an RNA blotting technique and have determined the relationship of this domain to a metal-binding site that was identified by sequence analysis. Atomic absorption spectroscopy has been used to confirm the presence of zinc in the virion outer capsid as well as in purified σ3.

R21.50

INHIBITION OF LYMPHOCYTE PROLIFERATION AND OF HEMOPOIETIC COLONY FORMATION BY HUMAN CYTOMEGALOVIRUS (HCMV) IN VITRO. T.A. Rakusan, K. Rowlands, H.S. Juneja. Departments of Pediatrics and Internal Medicine, University of Texas Medical Branch, Galveston, Texas.

HCMV inhibits phytohemagglutinin (PHA) induced lymphocyte proliferation and formation of granulocyte-macrophage (GM) colonies in vitro by bone marrow (BM) or cord blood (CB)mononuclear cells (MNC), independently of total interferon induced.

We have examined the role of soluble mediators released by HCMV infected MNC. Normal human BM, CB and peripheral blood (PB) MNC were infected with HCMV(strain AD169 or recent clinical isolates). Conditioned medium was prepared after 3 days and its effects on PHA response of PB and CB MNC and on GM colony formation by BM and CB MNC were determined.

CB and PB conditioned media inhibited PHA response equally and to the same extent as the virus infection did. The effect of both virus and conditioned media on PB MNC was greater than on CB MNC. Similarly, the inhibition of GM colony formation was at least partially mediated by conditioned media. Colony forming cells from BM were more affected than the colony forming cells from CB. These findings suggest that the inhibition of PHA response and GM colony formation by HCMV is largely due to soluble mediators released by the accessory cells. The target cells in CB appear less sensitive to HCMV inhibition than the cells in BM or PB.

R21.51

TERATOGENICITY OF THE SEMLIKI FOREST VIRUS MUTANT ts22 FOR MOUSE FOETUSES. M. J. Mabruk, G. J. Atkins. Department of Microbiology, Moyne Institute, Trinity College, Dublin 2, Ireland.

A characteristic of togaviruses such as rubella virus, bovine viral diarrhoea virus and border disease virus is the ability to infect the developing embryo or foetus, and to produce abortion or teratogenic effects. We have utilised the alphavirus Semliki Forest virus (SFV, strain A7) as a model to study embryonic and foetal infection. Wild-type SFV is rapidly abortigenic when injected intraperitoneally into 8-day pregnant mice, although the mothers survive. The mutant ts22 produces teratogenic effects in a proportion of offspring.

In this study we have analysed further the teratogenic effects of ts22, with particular reference to the skeletal system. We have used alizarin red as a stain for bone and alcian blue for cartilage. Many foetuses infected by ts22 show developmental retardation, and this is reflected in incomplete ossification. Many foetuses also show open spine lesions. Thus infection of pregnant mice with SFV - ts22 may be a relevant model for human spina bifida, a congenital disease in which closure of the neural tube is incomplete.

R21.52

EFFECTS OF THYROID-STIMULATING HORMONE ON THE REPLICATION OF VESICULAR STOMATITIS VIRUS AND BOVINE PARAINFLUENZAVIRUS 3 IN OVINE THYROID CELLS. M. Petric[1], M. Vitale[2], M. Eggo[2] and G.N. Burrow[2]. Department of Virology[1], The Hospital for Sick Children, and Department of Medicine, University of Toronto[2], Toronto, Canada.

Sheep thyroid cells, in primary culture, can be morphologically and metabolically differentiated by the addition of Thyroid-Stimulating Hormone, TSH. To gain further insight into the effects of this hormonal minipulation on cell polarity, the replication of Vesicular stomatitis virus (VSV),and Bovine parainfluenzavirus 3 (BP3) was studies in these cells. These viruses are known to be respectively tropic for the basolateral and apical surfaces of polarized cells. The yield of VSV at 5 hours post infection was up to 100 times higher in the presence of TSH. In contract,the yield of BP3 was increased by up to 10 fold in the absence of TSH. An exposure to TSH of 2-4 hrs before infection was required to increase yield of VSV. Studies on the time course of virus infection by immunofluorescence microscopy revealed that TSH had a direct effect on the rate of appearance of virus antigen. These observations are consistent with the concept that TSH affects ovine thyroid cell polarity.

R21.53

HISTOPATHOLOGICAL CHANGES IN THE BURSA OF FABRICIUS AND IMMUNOSUPPRESSION ASSOCIATED WITH IBD VACCINE IN CHICKEN C.D. Ezeokoli, A.I. Nwannenna, E.A. Ityondo, J.U. Umoh; Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria.

The effect of the infectious bursal disease (IBD) live virus vaccine on the bursa of Fabricus (BF) and immune response of chicken was evaluated. A total of 350 chicks were used. Groups were vaccinated at 1, 7, 14 and 28 days of age with IBD vaccine, and were subsequently challenged with wild type IBD virus. The BF was examined for histological changes at regular intervals following vaccination. Other groups were further given Newcastle disease (NDV) vaccine, and later challenged with virulent NDV. Antibody levels to NDV were monitored.

There was significantly higher mortality rate in vaccinated than unvaccinated birds (P 0.01).after challenge. The mortality was highest in those vaccinated at 28 days of age (54.4%) compared with 7 days 25%) or controls (13.8%). BF from vaccinated birds showed marked lymphocyte depletion in the medullarly areas of the follicles.

In a second study, intraocular NDV (NDV-i/o) vaccine given at day old largely prevented the immunodepressive effect of IBD on NDV. Groups that received IBD vaccine on day 14 but no NDV-i/o suffered higher mortality (41.2%) and showed lower antibody response than controls which did not receive IBD vaccine (11.8%).

R21.54

ANTIGENIC VARIATION OF EQUINE INFECTIOUS ANEMIA VIRUS DURING PERSISTENT INFECTION. F.Fuller,S.Perry, C.Kolmstetter & L.Coggins. North Carolina State University, Raleigh,NC. USA.

Equine infectious anemia (EIA) virus is a non-oncogenic, retrovirus in the Lentivirus group which causes a persistent infection in horses. The disease is typified by periodic episodes of clinical illness characterized by fever,anemia, anorexia, and CNS depression, with associated high titer viremia. Distinct antigenic strains of EIA virus predominate in the serum of a single infected animal during different clinical episodes; these antigenic variants are presumably produced by mutation in the envelope gene of the virus. In order to study the molecular nature of the antigenic variation, we have cloned envelope gene sequences of the equine cell-adapted Wyoming strain of EIA virus. Because most of the proviral forms integrated into equine cell DNA are apparently defective, we clones cDNA made to RNA purified from infectious virus into pBR322. We have identified 49 clones carrying virus-specific inserts, ranging in size from 500 to 2500 bp. Analyses by restriction enzyme digestion suggest the clones may contain inserts which span the entire 8 Kb genome. Several clones containing envelope gene sequences were subcloned into M13mp18 and sequenced by the dideoxynucleotide chain-termination method. In addition, the cell-adapted strain of EIA virus was used to infect three ponies in a sequential fashion. Virus was isolated from these animals and several isolates obtained during febrile episodes were analyzed for changes in the envelope genes.

R21.55

ABROGATION OF AUTOIMMUNE DIABETES IN BB-RATS BY A LYMPHOTROPIC STRAIN OF VIRUS. P.L. Schwimmbeck, T. Dyrberg, M.B.A. Oldstone. Department of Immunology, Research Institute of Scripps Clinic, La Jolla, CA, USA.

About 80% of BB-rats spontaneously develop insulin dependant diabetes mellitus (IDDM) by 60-140 days of age. The disease is autoimmune, because transfer of lymphocytes from IDDM to non-diabetic animals results in disease, while immunosupression of BB-rats prevents IDDM. We report the prevention of IDDM by infection with a lymphotropic strain of lymphotropic choriomeningitis virus (LCMV-Clone 13) at the age of 30 days. Over 200 days after introducing LCMV the incidence of diabetes was 35% versus 81% in the uninfected controls ($p < 0.01$). Viable virus was needed, as UV-inactivated virus resulted in the same incidence of IDDM as untreated animals. LCMV infected BB-rats had neutralizing antibodies to LCMV, that occurred by 10 days after infection and persisted up to 200 days. By infectious center analysis and in situ hybridization, infectious virus and viral transcripts could be demonstrated in PBL up to 10 days after infection, whereas RNA extracted from other organs was negative by RNA dot blot-analysis. FACS-analysis showed a 70% reduction of pan-T (W3/13+), helper (W3/25+) and suppressor (Ox8+) - lymphocytes at 4 and 7 days after infection, but a return to normal levels after 14 days. We conclude that lymphotropic virus through its infection of lymphocytes suppressed autoimmune response required to cause IDDM.

R21.56

PATHOGENESIS OF EGG-ADAPTED AND FIELD STRAINS OF AVIAN ENCEPHALOMYELITIS VIRUS D.R. Shafren and G.A. Tannock Faculty of Medicine, The University of Newcastle, New South Wales, Australia.

The pathogenesis of the Van Roekel and a neurovirulent field strain of avian encephalomyelitis virus (AEV), an enterovirus in the Family Picornaviridae, was studied using antigen and antibody ELISA assays. A triple antibody capture ELISA procedure for antigen had a sensitivity of 200 pg for each strain; a double antibody sandwich procedure was used to detect serum antibody at a dilution of 1:100.

Following yolk-sac inoculation of 6-7 day old specific-pathogen-free (SPF) chick embryos with the Van Roekel strain, viral antigen could be detected first in the brain and then in myocardial tissues. A similar pattern of infection was noted with a field strain after intramuscular inoculation to 5 week old SPF chickens and the trophism for myocardial tissue is characteristic of certain Coxsackie B strains.

The patterns of spread of the field virus were studied after inoculation by the intramuscular, intracerebral and oral routes. Although no differences were noted in the levels of antibody induced, highest levels of faecal shedding occurred in birds administered virus by the oral route.

R21.57

IDENTIFICATION OF A REGION IN THE MALIGNANT FIBROMA VIRUS GENOME RESPONSIBLE FOR ALLOWING INFECTION OF RABBIT LYMPHOCYTES. Justin J. Mulloy, David S. Strayer, Patricia Byers, Katherine A. Laybourn, and Julian L. Leibowitz. Department of Pathology, Univ. of Texas, Houston, TX, 77030, U.S.A.

We examined the ability of Malignant Fibroma Virus (MV) and Shope Fibroma Virus (SFV) to infect rabbit lymphocytes. MV, a leporipoxvirus closely related to SFV, causes malignancies in rabbits and is profoundly immunosuppressive. SFV causes benign fibromas and does not replicate in rabbit lymphocytes. We transfered the ability to replicate in lymphocytes, a property of MV, by electroporating cloned BamHI restriction fragments of MV into SFV infected RK13 cells. The recombinants generated by electroporation were selected for their ability to replicate in rabbit lymphocytes. The presence of varying sized portions of MV BamHI fragment C (10.8kb) were confirmed in 35 of 71 recombinants examined by Southern blot hybridization. Restriction mapping of the recombinants reveal that a common 1.1kb region of the MV BamC fragment was present in all 35 recombinants which were analyzed. This region mapped 74kb from the left terminis of SFV. Reciprocal recombinants have been generated by transfecting the corresponding SFV fragment into MV into infected cells. These reciprocal recombinants are presently being evaluated for the ability to grow in rabbit lymphocytes. The results will help confirm the importance of the 1.1kb MV fragment in allowing the MV virus to replicate in lymphocytes.

R21.58

PATHOGENESIS OF HERPES SYLVILAGUS IN COTTONTAIL RABBITS. R.M. Hesselton, W.C. Yang, P. Medveczky, J.L. Sullivan, Dept of Peds & Pharm, Univ of Mass Med Sch, Worcester, MA USA.

Twenty-five seronegative cottontail rabbits were infected with Herpes sylvilagus. Twelve animals served as controls. All infected animals seroconverted within 7 to 14 days. Virus was isolated from blood mononuclear cells (7/7), spleen (4/4), bone marrow (5/6), thymus (3/5), lymph nodes (3/5), kidneys (7/7), lung (6/6), and liver (4/5) as early as 3 days post-infection. Virus was isolated from PBL, bone marrow and kidneys from animals infected more than 1 year earlier. Both linear and circular H. sylvilagus DNA were identified in blood mononuclear cells and thymus of 4/5 juvenile (14 wk old) animals examined. Infected animals showed leukocytosis, monocytosis and lymphocytosis with the appearance of atypical lymphocytes. Peripheral blood abnormalities peaked at 10-14 days post-infection, returning to normal by 28 days with the exception of persistent atypical lymphocytosis for more than one year post-infection. Histopathological changes seen in virus-infected juvenile rabbits included viral myocarditis (5/7), interstitial pneumonia (7/7), and myositis (6/7) with lymphohistiocytic proliferation characterizing the cellular infiltrates. Reactive hyperplasia and subsequent lymphocytic depletion of spleen and lymph nodes (7/7) with lymphoid hyperplasia in many nonlymphoid organs (7/7) was observed. H. sylvilagus infection in cottontail rabbits results in immunopathological changes similar to those observed in severe Epstein-Barr virus infection in humans.

R21.59

REPLICATION OF VARICELLA-ZOSTER VIRUS IN HUMAN PROMYELOCYTIC CELLS(HL-60)

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HL-60 cell can be differentiated to macrophage by treatment of phorbol-12-myristate-acetate(PMA). The study of infection of VZV to HL-60 cells was carried out to investigate the relationship between virus replication and cell differentiation. First, HL-60 cells were treated with PMA and cells were infected with VZV. The expression of viral antigen tested by immunofluorescence technique and virus growth in this cell were compared with in non-treated HL-60 cell. Viral antigen was detected only in treated HL-60 cells and also virus could grow in differentiated HL-60 cells. Second, VZV was adsorbed to undifferentiated HL-60 cells and divided into two portions. After incubation for 1 day at 37 C, one portion was treated with PMA and another was untreated. Then, both groups of cells were plated on the monolayers of human embryonic cells for infectious center assay. The number of infectious centers in treated cells were approximately 100 times higher than in non-treated cells.

These findings indicate that VZV can replicate in differentiated monocyte, and virus may only be harbored in the undifferentiated monocyte. These experiments using a macrophage like cell line may provide useful informations of systemic VZV infection in vivo.

R21.60

IMMUNOPATHOGENESIS IN CANINE DISTEMPER VIRUS INFECTION OF THE HAMSTER CNS. J. Kirk[1], G.I. Cosby[1,2], I. V. Allen[1], S. J. Martin[2]. Departments of Pathology (Neuropathology)[1] and Biochemistry[2]. Queen's University of Belfast, Belfast, U.K.

The relationship between the humoral immune response and the neurovirulence of large and small plaque isolates of the Onderstepoort strain of canine distemper virus have previously been described (Cosby *et al*, 1983, Arch Virol, 78, 201-210). In the present study we have examined the role of cell-mediated immune mechanisms in the central nervous system (CNS), in the pathogenesis of disease. Electron microscopic and immunohistopathological techniques have been used to demonstrate the interactions of immune system cells with infected parenchymal cells. The nature and extent of these interactions differ according to the stage (early, late) and type (acute, delayed, immunosuppressed etc) of infection induced. For example an early predominantly plasma cell response, evident in small plaque virus-infected hamsters, was replaced at the equivalent time in immunosuppressed small plaque virus-infected animals by an inflammatory infiltrate rich in lymphocytes and and macrophages but with few plasma cells. It was in this group particularly that apparently cytotoxic cell interactions were seen.

R21.61

BINDING OF BARLEY STRIPE MOSAIC VIRUS TO POLIMERIZED PIG BRAIN TUBULIN.

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Barley stripe mosaic virus / BSMV / is transmitted through seed and pollen and can induce cytological and genetic disorders. Virions have been observed to bind, among others, to microtubules. It seemed to be interesting to determine whether or not the tubulins are the responsible components for such binding. In order to determine this we employed polimerized and unpolimerized tubulin from pig brain. After an incubation of tubulins with virions os BSMV Norwich strain and Tobacco mosaic virus / TMV / as a control, the polimerized tubulin was sedimented. Fractions were then analyzed by gel electrophoresis. A considerable binding of BSMV but not TMV virions to polimerized pig brain tubulin was observed. The binding of BSMV to polimerized tubulin indicates that plant tubulins may have a role in virus transmission.

R21.62

ALPHAVIRUS REGULATION OF THE COMPLEMENT SYSTEM.

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Two alphaviruses, Sindbis and Ross River have been reported to have contrasting effects on the serum complement system. Sindbis activated the alternate complement pathway while Ross River virus inhibited cleavage of C3 via both the alternate and classical pathways.

The outcome of the interaction of these two viruses with the complement system has been found to be determined by reversible conformational changes induced in the virus particle by the procedure used for purification.

Sindbis virus purified on 5-50% W/V sucrose gradients activated the laternate complement pathway while that prepared on 5-50% tartrate gradients inhibited the cleavage of C3. Ross River virus purified on a similar tartrate gradient also inhibited C3 cleavage. Ross River virus prepared on a sucrose gradient had no measurable effect on the complement system.

R21.63

ALTERATIONS OF INFLUENZA VIRUS PROTEIN SYNTHESIS IN A MONONUCLEAR PHAGOCYTE DIFFERENTIATION SYSTEM.

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The ability of differentiating mononuclear phagocytes to support replication of influenza A virus was examined.A model system was established using HL60 cells (a promyelocytic line) treated with 12-O-tetradecanoyl-phorbol-13-acetate (TPA,10^{-8}M) The extent of differentiation was determined by morphology, α-naphtyl acetate esterase staining, and expression of Fcγ receptor by binding of ^{125}I-3G8 monoclonal antibody and opsonized erythrocytes. Control and TPA-treated cells were infected at a multiplicity of infection (moi) of 1 or 10 plaque-forming units (PFU)/cell. Infectious virus yield and hemagglutinin (HA) titers were determined at various times.

Twenty-four hrs post-infection (PI), a 15-fold decrease of infectious virus production was observed in TPA-induced cells as compared to controls ($0.35x10^4$ vs $4.75x10^4$PFU/10^6cells). In contrast, no significant change was observed in the amount of HA expressed at the cell surface (58-70%HA$^+$ cells) or released into the medium (HA titers of 1:4-1:16), suggesting a replication block not related to HA synthesis or surface expression.

Control and TPA-induced cells were pulse-labelled with ^{35}S methionine at 4 and 18 hrs PI, and the extent of virus-specific protein synthesis was examined by SDS-PAGE and autoradiography. Results of these experiments indicated differences in the amount of individual viral proteins synthesized and timing of virus-induced host cell protein synthesis shut-off.

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R21.64

GENERATION OF A VIRULENT STRAIN OF SIV/SMM DURING PASSAGE IN A PIG-TAILED MACAQUE.

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A highly virulent strain of simian immunodeficiency virus from sooty mangabey monkeys (SIV/SMM) was obtained from tissues of a pig-tailed macaque (P5127) that had been inoculated with the virus 14 months earlier. In contrast to other macaques experimentally infected with SIV/SMM, P5127 developed antibodies only to env region gene products and never to gag region-encoded proteins. P5127 was sacrificed at 14 months postinfection because of chronic diarrhea, lymphadenopathy, splenomegaly, lymphopenia, thrombocytopenia, anemia, ataxia, and emaciation. Five of six macaques that received either 10 ml of blood just before sacrifice of P5127 or cell-free virus from lymph node tissue died within 8 days of infection. The one surviving animal has presented with diarrhea, weight loss, anemia, thrombocytopenia, and oral candidiasis. Histopathology showed lymphoid necrosis and large numbers of multinucleated giant cells in lymph nodes and other tissues in all animals that died. In addition, virus was isolated from multiple tissues, including brain. Biologic and molecular comparisons of SIV/SMM originally injected into P5127 and the apparently lethal variant obtained 14 months later are in progress.

R21.65

Cloning and mapping of bovine encephalitis herpesvirus DNA.

D.N. Bulach and M.J. Studdert, School of Veterinary Science, The University of Melbourne, Parkville, Victoria, Australia, 3052.

Bovine herpesvirus 1 (BHV1: infectious bovine rhinotracheitis (IBR) virus) is frequently mentioned though infrequently confirmed as a cause of encephalitis in calves. For Australia and Argentina it has been shown that a related but clearly and consistently different virus causes encephalitis (Bagust 1972; Studdert and Brake 1985; Metzler et al.1986). The most persuasive evidence that two distinct viruses are involved is based on restriction endonuclease DNA fingerprints.

We have cloned the Bam HI fragments of the prototypic bovine encephalitis herpesvirus strain N569 into pUC19 and E. coli JM83 and generated some restriction maps additfonal to those already published by Engles et al. 1986. Data will be presented exploring, by Southern blot hybridization, the relationships between BHV1, bovine encephalitis herpesvirus, a new ruminant herpesvirus isolated from buffalo and previously reported as BHV1 and caprine herpesvirus.

We argue that bovine encephalitis virus is totally different from BHV1 and hypothesise that the virus causes encephalitis in European cattle (Bos taurus) following transmission from Zebu/Brahman cattle (Bos indicus).

R21.66

CORONAVIRUS-INDUCED CARDIOMYOPATHY.

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Rabbit coronavirus (RbCV) was first encountered in testicular suspensions from rabbits infected with Treponema pallicum. RbCV infection produces fever, uveitis, myocardiopathy and death characterized by right ventricular hypertrophy, myocarditis, and pleural effusion. Blood from infected rabbits exhibited decreased platelet and RBC counts, hematocrits, and hemoglobin levels. Leukopenia followed by leukocytosis with a left shift was seen. Prothrombin time increased from 9.1 sec. to > 150 sec. 4 days following infection. Serum creatine kinase levels increased as much as 217 times pre-inoculation values on day 4 post infection. RbCV induces CF antibody to human coronavirus 229E. By PIA, RbCV is also related CCV, TGEV, and PEDV. This suggests that RbCV is a Group 1 coronavirus. RbCV and 229E have a bouyant density of 1.19 - 1.20 g/ml and their nucleic acids cross-hybridize with TGEV cDNA probes. Purified RbCV is infectious and appears as a coronavirus particle by EM. Antiserum to 229E, CCV, and FIPV and to a lesser degree TGE partially blocked the clinical course of the disease. These data strongly support a coronavirus etiology for the disease.

R21.67

DICHOTONOMY OF HTLV-I SPECIFIC IMMUNE RESPONSE IN ADULT T CELL LEUKEMIA/LYMPHOMA AND HTLV-I ASSOCIATED MYELOPATHY. S.Sonoda, S.Yashiki, K.Takahashi, M.Tara, M.Matsumoto, K.Usuku, M.Osame and A.Igata. Department of Virology, Internal Medicine, Cancer Research Institute, Faculty of Medicine, Kagoshima University, Kagoshima, Japan.

Adult T cell leukemia/lymphoma(ATLL) and the newly defined neurological disease, HTLV-I associated myelopathy(HAM) are caused by the same viral etiologic agent, HTLV-I but with different mode of pathogenesis being implicated between two.

In order to eluciate the diffence of immunopathology involved in ATLL and HAM, we investigated HLA-linked T cell immune response against HTLV-I using peripheral blood samples of 36 ATLL and 44 HAM patients.

ATLL patients had HLA haplotypes including HLA components of A26Bw62Cw3DR5DQw3 and exhibited a low response of T cell proliferation against HTLV-I virion antigen. In contrast, majority of HAM patients shared one of the unique HLA haplotypes, A24Bw52Cw-DR2DQw1, A11Bw54Cw1DR4DQw3 and A24B7Cw7DR1 DQw1, and exhibited a high T cell response against HTLV-I. Both low and high responder haplotypes were found in normal population and segregated with their T cell responses against HTLV-I as those seen with ATLL and HAM patients. These results indicated that ATLL and HAM patients have a different immunogenetic background for disease susceptibility associated with HTLV-I specific T cell immune response.

R21.68

IMMUNOSUPPRESSION INDUCED BY A MEASLES VIRUS ts MUTANT. S. Vydelingum, R. Marusyk & A. Salmi. Department of Medical Microbiology and Infectious Diseases, Faculty of Medicine, University of Alberta, Edmonton, Canada.

Immunosuppression-induced by measles virus (MV) infection has been known for almost a century but the mechanism(s) involved have yet to be determined. Recently, a temperature- sensitive isolate of MV (ts38) has been characterised in our laboratory and used to study measles virus immunosuppression in human peripheral blood lymphoid cells and subpopulations *in vitro*. By immunofluorescence and plaque assay studies, we have shown that progeny MV ts38 is not produced at 37°C though viral proteins are synthesized. The viability of cells infected with MV ts38 was not disturbed thereby excluding the cell death as a reason for immunosuppression in this system. MV ts38 abrogated mixed lymphocyte reaction and lymphocyte blastogenesis induced by mitogen or antigen *in vitro* almost as efficiently as the wild type MV. Cell fractionation experiments showed that MMA-marker positive adherent cells play a more important role in the inhibitory process than OKT3 positive T cells. However, the maturation of monocytes into macrophages *in vitro* had no effect on the inhibition. Using several MV strains as well as MV ts38 we have also shown that the inhibition can be transferred by the supernatants from infected lymphocytes. The release of IL-2 was not affected by infection with MV ts38 and interferon-alpha (IFN) was produced and released into the supernatant. As only a part of the inhibitory effect was reversed by antibodies to IFN, the bulk of the inhibition is mediated by molecules other than those studied. This nondestructive virus-cell system will be used in the further characterization of such macromolecules.

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R21.69

PERSISTENT AND ABORTIVE INFECTIONS OF HEMATOPOIETIC HUMAN CELL LINES BY VACCINIA VIRUS. B.G.-T. Pogo, A.C-K. Lai, M.E. Joesten, J.G. Holland and C. Friend. Center for Experimental Cell Biology and Department of Microbiology, Mount Sinai School of Medicine, New York, N. Y. 10029.

We previously reported that vaccinia virus can produce persistent infections in Friend erythroleukemia (FL) cells. We have now investigated the replication of vaccinia virus in human hematopoietic cell lines. HL_{60}, a promyelocytic cell line that can be induced to differentiate into either polymorphonuclears or macrophages; K562, a cell line originated from myelogenous leukemia that can differentiate into the erythroid pathway and NB104, a lymphocyte cell line, were infected with vaccinia virus at different multiplicities. The results indicated that HL_{60} cells do not support viral replication but are abortive infected, while the K562 and NB104 cells can be productively infected. Whereas NB104 were readily lysed, cells from the K562 line can be persistently infected as have been the FL cells. As in the case of the vaccinia FL cells, a high degree of spontaneous differentiation was observed in K562 cells persistently infected. Data will be presented that support the concept that viruses can cause disease not only by destroying cells but also by modifying the functions of the infected cells.

R21.70

EVIDENCE FOR A GENETIC FACTOR IN THE VIRAL-INDUCED ANTI-ACTIN RESPONSE IN MICE. W. Taechaudomtavorn and D.L.J. Tyrrell. Departments of Biochemistry and Medical Microbiology and Infectious Diseases, Faculty of Medicine, University of Alberta, Edmonton, Alberta, Canada.

Actin is a highly conserved cytoskeletal protein. Considerable difficulty has been encountered producing polyclonal antibodies to undenatured actin. However, the preliminary studies demonstrated that anti-actin antibody production can be enhanced by coimmunization with paramyxovirus matrix (M) protein. Balb/c mice immunized with actin which has interacted with M protein produced high titres of anti-actin antibodies in 30 of 31 animals, whereas mice immunized with actin alone produced lower titres of anti-actin antibodies in 10 of 35 animals.

Enhancement of anti-actin antibody production by M protein associated with the major histocompatibility complex class II antigens (H-2 haplotypes). $H\text{-}2^b$ strain (C57Bl/10J) was a high responder whereas $H\text{-}2^k$ (C3H/HeJ) strain and $H\text{-}2^d$ (Balb/cJ) strain were low responders. This finding suggested that there may be a "carrier-like effect" of M protein stimulating T-helper cells in high responder mice and subsequently enhancing the antibody production. This may be an important mechanism by which viruses can initiate autoimmune responses.

R21.71

ETIOLOGIC STUDIES OF HEMORRHAGIC FEVER WITH RENAL SYNDROME (HFRS) VIRUS IN SHANGHAI. Xiong Qi Hua and Fang Guo Fu. Department of Virology, Shanghai Hygiene and Anti-Epidemic Centre, Shanghai, China.

Twelve strains of HFRS virus were isolated directly from the sera of 16 patients. The sera were collected in a Shanghai county hospital during the same epidemic season. The positive rate of viral isolation was 75%, and all sera used for viral isolation were positive for HFRS virus specific IgM and IgG antibody. HFRS virus could be isolated from serum even though the titer of HFRS virus IgG was as high as 1:1280 by IFA test ten days after disease onset. All 12 isolates were passaged in mice.

In the Shanghai area, clinical symptoms of HFRS virus infection vary from slight to severe. Before the HFRS epidemic peak, HFRS virus antigen was detected in the lungs of the rodents *A. agrarius*, *M. musculus*, *R. favipectus*, *R. norvegicus* as well as the Insectivora *S. araneus*. HFRS virus transmitted from different rodents may cause varied human infection.

The viral strains isolated were analyzed by polyclonal and monclonal antibodies prepared against the Hantaan virus strains 76-118, R22 (*R. norvegicus* China), 82010H (*A. Agrarius* China), A9 (*A. agrarius* China) and BC1 (rat, Japan). We found many differences among the strains. Based on monoclonal antibody reactivity, the isolated strains could be divided into three subgroups: 1. antigenically similar to the 76-118, A9 and Chen (patient serum derived) strains; 2. antigenically similar to the R22 strain; 3. antigenically similar to the Chen , A9 or R22 strain.

R21.72

Role of the Epstein-Barr Virus (EBV) gp350 Envelope Protein in Attachment to the EBV/C3d Receptor (CR2) of B Cells. G.R. Nemerow, J. Tanner, Y. Wang, E. Kieff, R. Houghten and N.R. Cooper. Department of Immunology, Research Institute of Scripps Clinic, La Jolla, CA.

Studies were carried out to examine the role of the major EBV envelope protein, gp350, in mediating virus binding to CR2. Isolated gp350 from the B95-8 EBV producer cell line bound to purified CR2 in dot blot immunoassays or to CR2 bearing B cells when incorporated into liposomes. The binding of gp350 to CR2 was specific as it could be blocked by certain monoclonal antibodies to CR2 or to gp350. Since a computer generated comparison of the gp350 amino acid sequence with that of C3d sequence revealed two distinct regions of homology, studies were carried out to determine whether these regions represented CR2 binding domains. A 14mer synthetic peptide representing the gp350 sequence corresponding to one of the gp350/C3d homologous regions showed dose dependent binding to CR2 and inhibited EBV binding in an ELISA assay. Minor changes in amino acid sequence abrogated these properties. In parallel experiments a series of recombinant "anchorless" gp350/220 molecules were also used to probe receptor binding domains. These results suggest that a discrete epitope of homologous primary amino acid sequence in gp350 and C3d mediates binding of two unrelated molecules to CR2.

R21.73

Interferon Does Not Mediate the Resistance of SJL/J Mice to Street Rabies Virus (SRV) Infection. Donald L. Lodmell, Danny L. Wiedbrauk, and Larry C. Ewalt. NIH, NIAID, Lab. of Persistent Viral Diseases, Rocky Mtn. Labs, Hamilton, MT USA

Previous studies from our laboratory have shown that resistance of SJL/J mice to intraperitoneally-inoculated SRV was associated with the failure of the virus to ascend from the spinal cord to higher levels of the central nervous system (CNS). Here we report on the possible role of interferon produced in the CNS on this resistance. Following inoculation of resistant SJL/J and susceptible A/WySnJ mice, brains, spinal cords (SC), cerebrospinal fluids (CSF) and sera were harvested. Low titers of virus were detected in the SC and brain of A/WySn mice on the fifth day, but INF was not present in these tissues or the CSF. At this time, neither INF nor virus was present in SC, brain, or CSF of SJL/J mice. By the seventh day high concentrations of virus and interferon were present in SC and brains of A/WySn mice; the CSF was negative for virus, but contained INF. In contrast, minimal amounts of virus and little, if any, INF was present in CNS tissues or CSF of SJL/J mice. By the tenth day maximum concentrations of virus and INF were present in CNS tissues of A/WySn mice, whereas similar tissues of SJL/J mice were negative for virus and INF. INF was never detected in the sera of mice used in these experiments. Thus, there was no correlation between resistance of SJL/J mice to ascending infection in the CNS and the local or systemic expression of interferon.

R21.74

Pathogenic and Genotypic Characterization of Avian Reoviruses. Maurice C. Kemp, F. Dustan Clark, Sahara A. LeSage, Ellen W. Collisson. Dept. of Microbiology and Parasitology, Texas A&M University, College Station, Texas, U.S.A.

Avian reoviruses are the demonstrated causative agents of a variety of diseases in chickens. Based on the electrophoretic mobility of the genome segments of six avian reoviruses, we have shown that the viruses can be readily subdivided into two distinct genotypic groups, with minor differences allowing total differentiation of each virus within a group. Members of group I have been shown to repeatedly cause tenosynovitis and, to a lesser extent hepatomegaly. Members of group II, while exhibiting similar genotypic properties can be subdivided into three different pathotypes. All three of the group II viruses when administered orally induce malabsorption syndrome but, when adminstered via the footpad route, one virus induces a moderate tenosynovitis with minimal hepatomegaly. Another of the group II viruses does not cause tenosynovitis even at extremely high dose levels and a third group II virus causes severe tenosynovitis accompanied by gross pericarditis and myocarditis.

In addition to the characteristic genotypic pattern the group II viruses have been shown to cause the expression of a protein in infected cells that is not detectable in group I infected cells.

These results will be discussed relative to our efforts to determine the role of individual genes in the induction of specific disease states.

R21.75

SUSCEPTIBILITY OF MACROPHAGES TO INFECTION WITH MURINE CYTOMEGALOVIRUS IS H-2 DETERMINED

Patricia Price, J. Winter and G.R. Shellam, Dept. of Microbiology, University of Western Australia, Nedlands, Western Australia.

The resistance of mice to lethal infection with murine cytomegalovirus (MCMV) is known to be regulated by H-2 - associated genes, the H-2^k haplotype conferring resistance whilst the H-2^b and H-2^d haplotypes are associated with moderate or high susceptibility respectively. To a lesser extent non-H-2 genes also influence resistance to this virus. In order to study susceptibility to infection at the level of the individual cell without the confounding influence of the immune response in vivo, peritoneal macrophages from normal mice were infected with MCMV in vitro by centrifugal enhancement and were maintained in culture for 4 days. The infection was monitored by the expression of viral structural antigens, and by changes in cellular morphology. The effect of infection on the expression of Fc receptors and the uptake of colloidal gold by macrophages was also studied. The frequency of infected cells was 90% with H-2^d donors (BALB/c and B10.D2), 70% with H-2^b donors (BALB.B and B10) and 5% with H-2^k donors (BALB.K, B10.BR and CBA). Morphological and functional changes in the macrophages paralleled the expression of viral antigen and were stable between days 2 and 4 post infection, whilst cells remained viable and metabolically active. However no non-H-2 determinants of resistance were evident in the macrophages in contrast to observations in vivo. CMV DNA was detected only in H-2^b and H-2^d cells suggesting that the infection of H-2^k cells is limited at a very early stage. Since anti K^bD^b monoclonal antibody blocked the infection of B10 cells, these data are consistent with the notion that susceptibility may be due to the association of H-2^b or H-2^d antigens with the MCMV receptor.

R21.76

GENOMES OF SLOWLY CYTOPATHIC EQUINE HERPESVIRUSES

G.F. BROWNING AND M.J. STUDDERT, SCHOOL OF VETERINARY SCIENCE, UNIVERSITY OF MELBOURNE, VICTORIA 3052, AUSTRALIA.

The genomes of 51 slowly cytopathic equine herpesviruses were examined by restriction endonuclease digestion and Southern blotting. DNA of 47 viruses showed considerable fragment pattern heterogeneity, although some fragments co-migrated. 15 of the 47 viruses showed over 80% homology by Southern blot hybridization. 4 viruses, from 3 epidemiologically unrelated occurences, shared comigrating fragments only with each other and showed reduced homology to an EHV 2 strain; these 4 viruses appear prototypic of a novel, second equine betaherpesvirus. 25 of the viruses examined were from two epidemiologically linked cohorts - 3 yearlings isolated from other horses for 418 days, and 7 mares and their foals sampled monthly for 6 months after parturition. Although repeated isolation of the same virus for 54 days demonstrated persistent infection with a single virus, most repeat isolations yielded different viruses. Isolation of the same virus from different sites, indicated lack of site specificity and 2 viruses established latent infections with intermittent shedding. Multiple, concurrent local infection was demonstrated by the isolation of 2 or more viruses from the same nasal swab. Preliminary cloning and mapping of the approx. 190kb EHV 2 genome indicates that it does not isomerise. The genome of the novel virus is estimated to be approx. 149kb.

R21.77

COMPARATIVE STUDIES ON EXPERIMENTAL HERPESVIRUS LATENCY. J. Rajčáni, D.Blaškovič, M.Kúdelová, M. Kočišová, J.Svobodová. Institute of Virology, Slovak Acad. Sci., and Chair of Virology, Fac. Natural Sci., Comenius University, 817 03 Bratislava, Czechoslovakia

Two different models of herpesvirus persistence are described: that of human herpes simplex virus /HSV/ in rabbits and that of murine herpesvirus /MHV/ - a new member of family Alphaherpesvirinae - in mice. In more than 100 rabbits kept for upto 580 days after corneal inoculation, the results of direct examination of the cornea, both Gasserian ganglia and brain stem were compared with those of explantation. The formation of HSV-specific antigens was followed using anti-virion serum and a serum to nonstructural polypeptides. Viral DNA sequences were searched in cultured and noncultured tissues by two hybridization techniques. The results point at static latency in the ganglion in the absence of specific antigen formation. The positive hybridization in the brain stem was not in accordance with the explantation results.

Persistence of MHV in the lungs, spleen and kidney was of dynamic type being in sharp contrast with the static latency outlined above. Unlike to HSV, the MHV spread mainly by hematogenous route.

R21.78

FIXED AND STREET RABIES ELECTROPHYSIOLOGICAL ALTERATIONS IN EXPERIMENTAL RABIES. P. Gourmelon, D. Briet, L. Court, H. Tsiang, Institut Pasteur, Département de Virologie, Unité Rage, 25, rue du Dr. Roux 75724 Paris Cédex 15, France.

Changes in spontaneous brain electrical activity and sleep organization were investigated in mice and cats. Animals were chronically implanted with electrodes and EEG recording were monitored during all the evolution of the disease. Quantification of the brain electrical activities was evaluated using spectral analysis techniques.

In fixed rabies, drastic collapse in the organization of sleep stages was associated with brain electrical activity disturbancies. The main features of these abnormalities were: 1/ the disappearance of rapid eye movement sleep (REM); 2/ the regular and monotous slowing of the EEG activities (2-4 c/s); 3/ the constant occurrence of paroxysatic events. Cerebral death preceded cardiac arrest of about 30 minutes (Brain Res. 1986, 398, 128-140). In street rabies the EEG disturbancies were rare and sub-normal until the terminal phase. Paroxysatic elements were prevalent and contrast with the absence of disorganization of the brain electrical activity. Several pictures of sleep stage alterations were observed, mainly REM sleep modifications and total insomnia.

(This investigation was supported by grants from MRI N° 82.L. 1107 and INSERM, CRE N° 846019).

R21.79

HISTOPATHOLOGICAL CHANGES PRODUCED BY CBA MICE EXPERIMENTALLY INFECTED WITH PICHINDE VIRUS

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The organ distribution and pathogenicity of pichinde virus were studied in CBA mice following intracerebral and intraperitoneal inoculations. There was an age related response in CBA mice to pichinde virus infection. Suckling mice were found to be more susceptible to lethal pichinde virus infection. The virus multiplied to higher titres in the brain and liver of suckling mice. Histopathologic changes on tissues taken from moribund animals consisted mainly of inflammatory cell infiltrations and coagulation necrosis of liver, kidneys, brain and the spleen with marked eosinophilic amorphous debris in the white pulp of spleen and proximal convoluted tubules of kidneys. There was evidence of extra medullary haematopoiesis and large numbers of megakeryocytes in the spleen. The brain was congested with oedematous choroid epithelium and marked astrocytic and microglial reactions. The leptomeninges showed mild infiltrations with mononuclear cells mainly lymphocytes.

R21.81

MUTATION WITHIN THE PROTEIN BINDING SITES OF THE ENHANCERS WHICH DO NOT AFFECT THYMOTROPISM MAY ALTER THE THYMOMA INDUCING POTENTIAL OF MoMuLV. Yuen, P.H., P. Szurek, T.C. Suen, The University of Texas System Cancer Center, Science Park-Research Division, Smithville, TX.

Chimeric constructs generated by exchanging homologous fragments between the high thymoma inducer, MoMuLV and its weak thymoma inducing variant, MoMuLV-TB, localized the determinant critical to thymoma induction to 47nt encoding p15E, the noncoding 3' sequence and LTR. The total number of nucleotides constituting this fragment differ only in 3 single base insertions and 1 single base deletion. Thus, difference in the mode of action of this fragment is unlikely to be due to differences in the spatial relationship among the regulatory elements found within this fragment. Most of the mutations were found within the nonleukemia virus specific protein binding sites of the enhancers. This is consistent with the thymoma inducing potential. It is likely that binding of specific nuclear proteins to one or more of mutated binding sites may be altered which consequently affected the thymoma inducing potential of MoMuLV-TB. MoMuLV-TB or the recombinant viruses ith MoMuLV-TB LTRs were also shown to replicate in TB cells or in thymocytes as efficiently as MoMuLV or the recombinant viruses with MoMuLV LTR, suggesting that thymotropism per se cannot account for the thymoma inducing potential of MoMuLV.

R21.83

HUMAN ANTIBODY RESPONSE TO NONSTRUCTURAL PROTEINS OF DENGUE VIRUS DURING INFECTION. L. Corner, Y.C. Chan, M.L. Ng and E.H. Yap. Department of Microbiology, Faculty of Medicine, National University of Singapore, Lower Kent Ridge Road, Singapore 0511, Republic of Singapore.

Dengue fever (DF) and its more severe form, dengue haemorrhagic fever/dengue shock syndrome (DHF/DSS), is an important public health problem in Southeast Asia. It has been postulated that the humoral immune response of the host influences the severity of dengue disease. Dengue virus-infected cells contain several nonstructural, virus-specified proteins in addition to the virion envelope glycoprotein E and capsid protein C. To further clarify the role of antibody in the pathogenesis of dengue infections, the Western blot technique was used to identify the dengue virus proteins which elicit an immune response in the host. Antibodies to the three large nonstructural proteins, NS1 (GP48), NS3 (P67) and NS5 (P98) of dengue-2-infected C6/36 cells were observed in acute- and convalescent-phase sera of DF and DHF patients. In some patients, antibody to either NS1 or NS3 alone was detected. The sera reacted most frequently with NS3 and antibody directed against it generally appeared earlier than that of NS1. Antibody rises to both NS1 and NS3 were demonstrated from the increased intensity of the stained bands seeen in the convalescent-phase sera.

R21.80

Identification of bovine leukemia virus (BLV) infected cattle by ELISA employing monoclonal antibodies directed against BLV-p24. G.F. de Boer, Central Veterinary Institute, Lelystad, the Netherlands.

Six hybridoma cell lines which secrete antibodies against bovine leukemia virus (BLV) core protein p24 have been established. The monoclonal antibodies (MCA) are directed against three antigenic determinants of BLV-p24. Various enzyme-linked immunosorbent assays (ELISA) were tested for the applicability of these six MCAs in BLV antibody detection. A highly sensitive IDAS- ELISA detecting antibody against BLV-p24 was developed, but aspecific reactivity made this test unsuitable for enzootic bovine leukosis (EBL) screening. The non-specific reactions were eliminated by a complex-trapping-blocking (CTB)-ELISA in which anti-BLV-p24 coated plates are simultaneously incubated with undiluted serum and FLK BLV antigen. Without washing, HRPO conjugate prepared of a different anti-BLV p24 MCA is added and further incubated. The CTB-ELISA cannot be used for screening of milk samples due to lack of sensitivity. The test results obtained with sera, however, matched remarkedly well with those obtained with AGPT and ELISAs detecting antibodies against BLV-gp51. In serially collected serum samples from BLV-infected calves antibodies were demonstrated at about the same time by CTB-ELISA-p24 and AGPT-gp51. The CTB-ELISA can replace the AGPT in EBL screening programmes utilising serum samples.

R21.82

ORGAN SPECIFIC SELECTION OF VIRAL VARIANTS DURING PERSISTENT INFECTION. Chwan-Chuen King[1], & Rafi Ahmed[2], Division of Epidemiology, UCLA School of Public Health[1] and Department of Microbiology & Immunology, UCLA School of Medicine[2], Los Angeles, CA 90024.

There is organ specific selection of viral mutants during persistent lymphocytic choriomeningitis virus (LCMV) infection of mice. LCMV isolates with different biological properties are present in the central nervous system (CNS) and lymphoid tissues of individual carrier mice. LCMV isolates from the CNS induce potent virus specific T cell responses in adult mice and the infection is rapidly cleared. In contrast, LCMV isolates derived from the lymphoid tissues of carrier mice suppress virus specific T cell responses and cause persistent infections in adult mice. We have identified the cell types in the lymphoid tissues of carrier mice in which variants are selected. Our results show that infection is confined primarily to T cells of the helper subset ($L3T4^+$) and macrophages, with minimal involvement of cytotoxic T cells ($Lyt2^+$) and B cells. LCMV isolates have been obtained from highly purified populations of macrophages and T helper cells and variants with the ability to persist in adult mice can be isolated from both cell types. Our results show the importance of host organs and cell types in the selection of viral variants.

R22.1

H.P.V. 16 et 18 DANS LES CANCERS GENITAUX, LES C.I.N. ET LES LESIONS BENIGNES (A PROPOS DE 134 CAS). A. BENDIB(1), A. BOUGUERMOUH(2), A. CHOUITER(3), A. ALLOUACHE (1). Service de Chirurgie(1) et de Cytopathologie(3), Centre Pierre et Marie Curie d'Alger ; Service de Virologie(2), Institut Pasteur de Sidi Fredj, Algérie.

Depuis 1984, 134 malades présentant 70 cancers invasifs du col, 20 C.I.N., 39 lésions bénignes (cervicites essentiellement), 3 cancers de la vulve, 1 cancer du vagin et 1 cancer du clitoris ont bénéficié d'un prélèvement biopsique qui a été traité par technique d'hybridation moléculaire à la recherche des virus H P V 6, 11, 16 et 18.
L'H P V 16 et 18 ont été retrouvés, dans plus de 50% des cas dans les carcinomes épidermoïdes invasifs du col et de la vulve, dans plus d'1/4 des cas dans les C.I.N. et dans 5% des cas dans les lésions bénignes.
L'H P V 16 a été retrouvé sur un ganglion de Leveuf et Godart et un ganglion lombo-aortique chez la même malade.
La présence d'H P V 16 ou 18 semble varier avec le stade clinique, la durée de la période d'évolution, la différenciation histologique et le taux d'hémoglobine dans le sang.

R22.2

PRESENCE OF HUMAN PAPILLOMAVIRUS AND HERPES SIMPLEX VIRUS TYPE 2 DNA SEQUENCES IN MALIGNANT AND NONMALIGNANT LESIONS OF CERVIX UTERI., N. Manjunath, H.Kaur, S.Bala and P.Seth, Department of Microbiology, All India Institute of Medical Sciences New Delhi. Biopsy tissues from dysplasias and carcinoma of the uterine cervix were examined for the presence of human papillomavirus (HPV) and Herpes simplex virus type-2 (HSV-2) specific DNA sequences. Bgl II/N fragment of HSV-2 (strain HV-219) was cloned in pKC-16 vector. Clones of HPV-16 and 18 in pBR-322 were obtained from Dr.L.Gissmann. DNA from cervical biopsies from 25 patients with invasive carcinoma of uterine cervix and 9 patients with cervical dysplasias were digested with various restriction endonucleases. The digested DNAs were Southern transferred and probed with ^{32}P labelled vector-free HPV-16, HPV-18 and HSV-2/Bgl II/N DNA. In 20/25 (80%) patients with invasive cervical carcinoma hybridization was seen with HPV-16 probe at Tm -40°C. When hybridization was carried out at Tm -18°C, 10 and 3 biopsies from cancer patients were positive for HPV-16 and HPV-18 DNAs respectively. In 7 biopsies, no hybridization was seen with HPV 16 and 18 probes at high-stringent conditions, suggesting that some other HPV type/s may be present. In 1/9 patients with cervical dysplasia hybridization was seen with HPV-16 probe at high stringent conditions. On the other hand with HSV-2/Bgl II/N probe, hybridization was seen with 1/15 biopsies from cervical carcinoma only. This biopsy was also positive for HPV-16 DNA.

R22.3

IDENTIFICATION OF THE TRANSFORMING GENES OF HUMAN PAPILLOMA VIRUS TYPE 16. L.Banks[1], J.Schneider[2], N.Jones[3], G.Matlashewski[4], K.Osborn[2], A.Murray[2] and L.Crawford[2].
1. Department of Biochemical Virology, The Wellcome Research Labs., Beckenham, Kent BR3 3BS, U.K.
2. Molecular Virology Lab., ICRF, London, U.K.
3. Gene Regulation Lab., ICRF, London, U.K.
4. Institute of Parasitology, MacDonald College, McGill University, Quebec, Canada.

Human papillomaviruses (HPV) have been implicated as etiological agents in the development of malignant lesions of the genital tract. In particular HPV-16 is closely associated with cervical carcinoma. It has recently been demonstrated that HPV-16 DNA can be used to transform cells in tissue culture. Hence we proceeded to identify the regions of the HPV genome essential for cell transformation.

A panel of HPV-16 deletion mutants were generated, lacking sequences in various open reading frames. These mutants were then assayed for their ability to transform cells. The results from these assays show that mutants deleted in the E1, E2, E4, L1 and L2 open reading frames are all capable of causing cell transformation. However, mutants deleted in the E6/E7 region of the genome are totally defective for cell transformation. Assays are now currently in progress to determine whether both E6 and E7 are required for cell transformation, or if either E6 or E7 alone are sufficient.

R22.4

ANALYSIS OF HPV-6vc cDNA SYNTHESIZED FROM A VERRUCOUS CARCINOMA. L.F. Roumillat, J.L. Patton, B.H. Devlin. Division of Viral Diseases, Center for Infectious Diseases, Centers for Disease Control, Atlanta, Georgia 30333

A human papillomavirus (HPV) type 6vc cDNA library was synthesized using polyadenylated RNA isolated from a rapidly growing vulvar verrucous carcinoma. The double-stranded cDNA's were cloned in BNN 45 E. coli using gt11 lambda phage as the vector. Recombinant HPV-6vc cDNA constructs were identified as complimentary to a cloned HPV-6vc-pBR322 constructed plasmid and then recloned for sequencing in a pUC 9 vector. HPV genes contained within the cDNA constructs were identified using HPV-6vc specific synthetic oligonucleotides. The HPV-6vc library contained predominantly a cDNA that was generated from RNA transcribed from an "early" HPV region. Results of this study will be described.

R22.5

TRANSFORMATION OF C127 CELLS AND MOUSE PRIMARY FIBROBLASTS BY PAPILLOMAVIRUS DNA. H. Sarkkinen, H. Kinnunen, H. Karjalainen, E. Ripatti, A. Ryhänen, T. Nurmi, S. Parkkinen, S. Syrjänen, K. Syrjänen, R. Mäntyjärvi. Department of Clinical Microbiology, Department of Oral Pathology; Institute of Dentistry, University of Kuopio, and Laboratory of Pathology and Cancer Research, Finnish Cancer Society, Kuopio, Finland.

DNA's of several animal papillomaviruses(BPV) induce morphologically transformed foci in continuous cell lines. Transformation of cultured cells with DNA of human papillomaviruses (Yasumoto et al., J. Virol. 1986, 57: 572) and transformation of primary cells either with animal or human papillomavirus DNA has proved more difficult. In the present study we transfected both C127 cells and mouse primary fibroblasts (C57BL/6J) with DNA's of BPV1, HPV 16 and HPV 31 by calcium phosphate coprecipitation (Graham and Van der Eb, Virology 1973, 52: 456). Foci of morphologically changed C127 cells were noticed 2 weeks (BPV) or 4-5 weeks (HPV 16 and HPV 31) after transfection. In contrast, morphological changes were not detected in C57BL/6J fibroblasts followed for 8 weeks. However, after several blind passages morphologically changed cells were detected in cultures transfected with BPV1. Foci of morphologically changed C127 cells or C57BL/6J fibroblasts were then collected, expanded, and analyzed for viral DNA by in situ hybridization, filter in situ hybridization and southern blotting. These results will be presented and discussed.

R22.6

NUCLEOTIDE SEQUENCE OF THE EUROPEAN ELK PAPILLOMAVIRUS (EEPV). A.Eriksson, H. Ahola, U. Pettersson* and J. Moreno-López.
Department of Veterinary Microbiology (Virology), Swedish University of Agricultural Sciences, Biomedical Center, Box 585 and *Department of Medical Genetics, Uppsala University, Biomedical Center, Box 589, S- 751 23 Uppsala, Sweden.

The genome of the European Elk Papillomavirus (EEPV) was determined to be 8095 nucleotide base pairs long. The genetic organization of the EEPV is similar to that of other papillomaviruses. Ten open reading frames (ORFs), designated E1-E7 and L1-L3, were identified, all of them located on one strand. Two possible polyadenylation signals were found, one is located in the non-coding region (NCR) preceding the early ORFs and the other, preceding the L2 ORF.

The EEPV genome shows a strong homology with the genome of the Deer Papillomavirus (DPV) and the regions of highest nucleotide sequence homology between EEPV and DPV were found in E1 ORF (70%), E5 ORF (69%) and L1 ORF (74%).

R22.7

Detection of human papillomavirus type 18 and herpes simplex type 2 DNA in a squamous cell carcinoma

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*Institute of Virology, "La Sapienza" University of Rome and Laboratory of Virology, Regina Elena Institute for Cancer Research, Rome -Italy.

There are strong suggestions that human papillomaviruses (HPV) are involved in genital tumors but additional agents might act as co-carcinogens in a multistep process leading to full malignant conversion. In this process herpes simplex type 2 (HSV2) might be involved through a "hit and run" mechanism as an initiating event.

We have found genomic sequences of HPV 18 and HSV2 in a DNA extracted from a vulvar carcinoma. Southern blot analysis have revealed that HPV18 DNA seems to be in an episomal form while HSV2 DNA is integrated in the host genome. The HSV2 integrated DNA corresponds to the Bgl N trasforming sequence of the virus genome. Although restricted at only one case, our findings seem to agree with the hypothesis that HSV2 and HPV play an ethiological role in the malignant conversion through a multistep process.

Work partially supported by A.I.R.C./86.

R22.8

HUMAN PAPILLOMAVIRUSES DETECTED IN THE MOUTH, LARYNX AND OESOPHAGUS. P.E. Gibson+ and M.S. Seymour*,+Virus Reference Laboratory, Central Public Health Laboratory, Colindale Avenue, London NW9 5HT U.K. *Dept. of Dentistry, Harefield Hospital, Harefield, Middlesex, U.K.

Papillomas of the oral cavity and larynx are known to be caused by HPV types similar to those found in the genital tract although malignant conversion seems to be a rare event. We have investigated gingival biopsies from several patients who developed hyperplasia of the gums following cardiac transplant. These patients had all been treated with cyclosporin which has been reported to cause gingival overgrowth as a side-effect to its anti-rejection action. From the gum of one of these patients HPV-16-like DNA was detected which differs in its restriction pattern from the prototype HPV 16.

We have also detected HPV types 6, 11 and 16 in laryngeal and oesophageal warts and biopsies. From one of the laryngeal specimens a unique HPV type was detected which does not cross-hybridize with HPV 6,11,16,18 or 31. HPV 6 was detected in the same specimen.

R22.9

TRANSFORMATION OF NIH 3T3 WITH HPV16 BUT NOT HPV6 DNA.

P.M. Chesters and D.J. McCance, Department of Microbiology, UMDS, Guy's Campus, Guy's Hospital, London. SE1 9RT

HPV16 and 6 were cloned into p6Vneo2 in both orientations with respect to the SV40 promoter and transfected into NIH 3T3 cells. The cells were selected for G418 resistance 48 hours later and resistant colonies which developed 10-14 days post selection were trypsinized, pooled and transferred from the 90mm dish to a 25sqcm flash. After the cells reached confluence they were seeded into soft agarose at 10^4 or 10^5 cells per 50mm dish. p6Vneo HPV16 in the 5' - 3' orientation produced large colonies after 14 days while in the 3' - 5' orientation colonies were much smaller. There were in both orientations 12 colonies per 1000 cells seeded. p6Vneo HPV 6 did not produce colonies when in either orientation and neither did p6Vneo2 alone or p6Vneo HPV1. C127 mouse cells using the same assay system did not produce colonies in soft agarose when transfected with any of the above constructions.

Sub genomic fragments of HPV16 have been cloned into p6Vneo2 and are being tested for the colony producing potential.

R22.10

A SURVEY ON THE INFECTIONRATE OF GENITAL PAPILLOMAVIRUSES BY BIOCHEMICAL ANALYSIS OF CERVICAL SMEARS.

E-M. de Villiers[1], D. Wagner[2], A. Schneider[3], C. von Knebel[4], H. Miklaw[4], H. zur Hausen[1]. [1]Referenzzentrum für humanpathogene Papillomviren, Deutsches Krebsforschungszentrum, Heidelberg; [2]Ev. Diakoniekrankenhaus, Freiburg; [3]Univ. Frauenklinik, Ulm; [4]Salem-Krankenhaus, Heidelberg; FRG.

We extended an epidemiologic study to biochemically assess the prevalence of genital HPV infections in women without cytological and colposcopic signs of nuclear atypias or dysplastic lesions. By including such smears from 8755 women (out of a total of 9295 patients) 9,3 % were found to hybridize with ^{32}P-labelled HPV 16/18 or HPV 6/11 DNA by using filter in situ hybridization. Presence of viral DNA correlated with age groups, i.e. between 15 and 50 years approximately 11 % were found to be positive. Thereafter the percentage of HPV-positive smears declined to about 5 %. An interesting difference appears to exist between onset of viral infections and the age distribution of women with cytological aberrations. Possible interpretations will be discussed.

In the course of these studies an apparently new virustype, relatively closely related to HPV 7, was isolated from a Bowenoid papulosis and characterized. This type has been found to be present in additional biopsies from Bowenoid papulosis or VIN investigated.

R22.11

THE REINDEER PAPILLOMAVIRUS (RPV): TRANSFORMING PROPERTIES CORRELATE WITH A HIGHLY CONSERVED E5 REGION.

J. Moreno-López, H. Ahola, A. Eriksson and *U. Pettersson.
Department of Veterinary Microbiology (Virology), Swedish University of Agricultural Sciences, Biomedical Center, Box 585, and *Department of Medical Genetics, Uppsala University, Biomedical Center, Box 589, S-751 23 Uppsala, Sweden.

A papillomavirus was isolated from a cutaneous fibropapilloma, on a Swedish reindeer. No sequence homology was detected between RPV DNA and the DNAs of bovine papillomavirus type 1 (BPV-1) and the avian papillomavirus (FPV) when hybridization was performed under stringent conditions. However, the RPV genome hybridized to the genomes of the European Elk Papillomavirus (EEPV) and the Deer papillomavirus (DPV) also under stringent conditions.

Selected regions of the RPV genome were studied by nucleotide sequence analysis. The E5 ORF of RPV has the potential to encode a 44 amino acid long which is similar to the E5 polypeptides of BPV-1, DPV, and EEPV. RPV is oncogenic for hamsters and transform C127 cells in vitro. Several virus specific mRNAs were detected in PVR-transformed C127 cells.

R23.1

PRELIMINARY STUDIES ON ANTIGENIC VARIATION OF POLIOVIRUS WITH NEUTRALIZING MONOCLONAL ANTIBODIES. Guo Ren,Tang Enhua,Wang Hong,Yang Xiaofeng,Liu Mingying,Qin Hongxing,Li Qihan,Zhuang Junying,Liu Kaimin. Institute of Medical Biology,Chinese Academy of Medical Sciences,Kunming,China.

With 5 groups monoclonal antibodies, the cross-neutralizing assays were done on 85 strains of type 1. According to the antigenic differences, these 85 strains of type 1 were classified into 10 subgroups. There were remarkable differences in the antigenicity between the 10 subgroups. Among these subgroups, the P1-2(28.36%) and the P1-5(43.24%) were dominant and have been epidemic ones in China in recent years. In the antigenicity, these two subgroups strains were different from Sabin-1 strain, but they shared common antigenic epitope with Mahoney strain and Brunhilde strain because of their responses to group C monoclonal antibodies. Similarly, the 91 strains of type 3 poliovirus were classified into 6 subgroups with other 5 groups of monoclonal antibodies. The results showed that the strain P3/Yunnan/2/84 which was isolated in poliomyelitis cases in local epidemic in Yunnan province of China,1984 and the strain P3/Finland/23127/84 which was isolated in Finland,1984 were different from the Sabin-3 strain, the reference virulent strain the their likes in the antigenicity.

R23.2

HVT REPLICATES IN FEATHER TIPS OF IMMUNIZED CHICKENS BUT FAILS TO PROTECT AGAINST CHALLENGE WITH A VIRULENT MDV FIELD ISOLATE. H.Levi*, I. Davidson**, M Malkinson** and Y. Becker*. *Department of Molecular Virology, Faculty of Medicine, The Hebrew University, Jerusalem, Israel. **Kimron Veterinary Institute, Beit Dagan, Israel.

The DNA of herpesvirus of turkeys (HVT) has only 4-6% homology with Marek's disease virus (MDV) DNA, but HVT vaccines protect chickens against lymphoma formation. HVT DNA was cloned in the plasmid pBR322 and related plasmids. To verify the viral nature of the cloned DNA fragment, the recombinant plasmids were hybridized to labeled cell DNA, and those showing negative results were tested for homology with purified HVT DNA. Twenty-seven recombinant plasmids were obtained, representing about 90% of the whole genome. These plasmids have served to identify the sequences homologous with MDV DNA and also as probes for detection of the virus in hybridization experiments with DNA extracted from infected cells and feather tips from immunized chickens.

The effectiveness of vaccination with HVT was evaluated by immunizing one-day-old SPF chickens with HVT and challenging half of the birds with a virulent strain of MDV nine days after injection of HVT. The remaining chickens were left in contact with the injected birds. Hybridization experiments by the dot-blot technique done with DNA extracted from the feather tips of these chickens, and the HVT recombinant plasmids as probes, revealed a very small amount of HVT DNA in the feather tips, appearing in the HVT-immunized birds from day 10 after vaccination, with a decline after 45 days. It was concluded that HVT is able to replicate in the feather tips, but this property did not suffice to protect chickens against challenge with a virulent MDV field isolate. Supported by U.S.-Israel Agricultural Research and Development Fund (BARD)

R23.3

EFFECT OF VACCINATION ON SPREAD OF MAREK'S DISEASE VIRUS (MDV) FROM FEATHER FOLLICLE EPITHELIUM. I. Davidson*, M. Malkinson*, C. Stronger* and Y. Becker**. *Avian Dept., Kimron Veterinary Institute, Beit Dagan. **Dept. of Molecular Virology, Faculty of Medicine, The Hebrew University, Jerusalem, Israel.

MDV is produced in the feather follicle epithelium (FFE), accumulates in the feather follicle space and feather tip cavity and spreads horizontally thorugh feathers and dander. MDV infection of chickens is monitored in feather tips. We present data on the level of MDV antigens in feather tips of chickens vaccinated with HVT, SB1, HVT+SB1 and Rispens (CV-1) vaccines. All groups were challenged with virulent Israeli field isolates. Appropriate infected non-vaccinated and non-infected controls accompanied each series. Each group included a challenge injected and in-contact infected group. Mortality and morbidity were recorded; MDV antigens in feather tips were determined by ELISA and AGP (I. Davidson et al., J. Virol. Meth. 13: 231, 1986; 14: 237, 1986). The level of MDV antigens measured by both methods was in complete agreement with the rate of protection rendered by the vaccine employed. As the efficacy of a vaccine increases (HVT+SB1 as compared with HVT and SB1 alone), the level of MDV antigens in the feather tips decreases. The vaccines also caused a delay in the appearance of antigens in the feathers of in-contact infected birds. The same situation was observed with the Rispens vaccine. The HVT and SB1 vaccines afforded moderate protection and the level of MDV antigens was somewhat lowered as compared to the non-vaccinated control group. All vaccinated birds showed elevated IgG in their feather extracts by the ELISA reaction with SPR serum. Non-vaccinated and birds challenged with virulent isolates did not show this phenomenon. Supported by U.S.-Israel Agricultural Research and Development Fund (BARD)

R23.4

EFFICIENCY OF A SOLID PHASE EIA IN ASSAYING EFFICACY OF ORAL POLIO VACCINATION (OPV) IN BANGLADESH. A.M.Z. Hussain and N.J. Marchette. Department of Trop. Med. and Med. Microbiol. University of Hawaii, Honolulu, Hawaii, USA.

A solid phase (polyvinyl) bound semi-crude polioviral antigens had been found to be 81.5%,80.8% and 84.2% sensitive with 90.4%,81.8% and 82.8% specificity for detecting serum antibody collected in filter papers after OPV with the three types of polioviruses respectively in comparision to a neutralization test result.

Neutralization test was inconsistent if sera were not shaken well before mixing with virus and EIA was inconsistent if washing was not optimal. 0.05 ml of serum was as efficient as 0.1 ml per test for neutralization as well as EIA tests. Sera eluted from filter paper seemed to have a non-specific delaying effect on viral activity. Enzyme reaction time in EIA varies depending on the temperature of substrate buffer and reaction environment and variability of sera samples. Sensitivity of EIA is better when serum is multitypic. Better discrimination is obtained in EIA when antigen or enzyme controls are used as blanks rather than the buffer blanks.

Sero-conversion after OPV has been found significant in this control trial;although triple sero-conversion was found in only 11% of vaccinees. Demographic,clinical and nutritional variables had insignificant effect on sero-conversion. Effect of other enteroviruses is being tested.

Natural infection is most common with poliovirus type 1 followed by type III in Bangladesh.

R23.5

STUDIES ON SOLUBLE COMPLEMENT FIXING ANTIGEN OF JAPANESE ENCEPHALITIS VIRUS.

Jagdish Rai Dept. of Microbiology Christian Medical College and Hospital, Ludhiana.(Punjab) India.

A native soluble complement fixing(SCF)antigen is found naturally occurring in Japanese encephalitis. (JE) virus-infected mouse brain and tissue culture preparations, after removal of the particulate virional antigens. JE SCF antigen, further purified, was studied in detail for its physico - chemical and immunobiological characters. Purified by gel chromatography and sucrose density gradient ultracentrifugation, this antigen gave a single protein band in polyacrylamide gel disc electrophoresis and had a molecular weight approximately 40-45 x 10^3. It was resistant to treatment with mercaptoethanol (2 - ME) and sodium lauryl sulphate(SLS) while the virionol complement fixing (CF) antigens,including sucrose acetone extracted antigens were unstable to this treatment. JE SCF antigen was more type/virus specific than the sucrose acetone extracted virionol antigens in CF and agarose - gel double diffusion (AGD) immunoprecipitation test. The employment of JE SCF antigen as a useful diagnostic reagent in CF and AGD tests for specific serodiagnosis of JE virus infection is recommended.

R23.6

DIFFERENTIAL ANTIBODY RESPONSE TO DENGUE VIRUS INFECTION IN RELATION TO VIRUS SEROTYPE. M.J. Cardosa & P.H. Tio, School of Medical Sciences, Universiti Sains Malaysia, Penang, Malaysia.

During an outbreak of dengue fever and dengue haemorrhagic fever in Malaysia during the months of April to August 1986, acute phase serum specimens from patients with suspected dengue virus infections were inoculated into cultures of a macrophage cell line sensitised with anti-dengue antibody. This method, based on the principle that macrophage Fc receptors mediate enhanced infectivity of flaviviruses in the presence of antiviral antibody ("antibody dependent enhancement" or ADE), yields virus isolates from greater than 80% of acute phase specimens. We have isolates from over 60 patients, and we have examined their acute and convalescent phase sera for antibody levels, comparing the titres of neutralising antibody with the titres of antibody capable of producing an ADE effect (enhancing antibody). The results of these studies are discussed in relation to the serotypes of the viruses isolated from each patient.

R23.7

THE ANTI-HBs RESPONSE AS DETECTED BY ELISA. M. Pecec, G. Stihi, N. Cajal. Immunochemistry Unit, S. Nicolau Institute of Virology, Bucharest, Romania

HBsAg purified by treatment with pepsin and detergent, bound to aminoalkyl glass or polyvinyl chloride and an anti-Fc-peroxidase conjugate form the assay system. By adjusting the dilution of the conjugate, linear log dose/response relationships are obtained through 0.5 - 200 ng IgG/ml, while the background remains under 0.05 u.e.

Titration curves of human sera are fitted according to Rodbard's four parameter equation and the affinity ($A = \sum n_i a_i \simeq \log K_0$) is computed from the fitted data, as a function of the antibody concentration. Every serum sample can be characterized by the values $A_{max}./2$ and the corresponding antibody concentration (C_0). Differences in specificity can be observed from the variation of the upper asymptote of the titration curves and by varying the composition of the solid phase antigen.

Results obtained with sera of subjects belonging to different population groups, are presented.

R23.8

PROTEIN A-GOLD (PAG) TECHNIQUE FOR THE DETECTION AND IDENTIFICATION OF RICE STRIPE VIRUS (RSV) BY IMMUNOELECTRON MICROSCOPY. Y. Honda, K. Ishikawa and T. Omura. National Agriculture Research Center, Tsukuba, Ibaraki 305, JAPAN.

The diagnostic capabilities of immunoelectron microscopy have been developed by using antibodies labelled with PAG. In comparative tests, the limiting concentrations for the detection of purified RSV were 2.2, 35.5 and 140 ng/ml by enzyme-linked immunosorbent assay (ELISA), PAG technique and latex flocculation (LF) assay, respectively. RSV was detectable in crude extracts from infected rice leaves at dilutions of 2,048,000-, 512,000- and 64,000-fold by ELISA, PAG technique and LF assay, respectively. RSV was also detectable in crude extracts from single viruliferous planthoppers, Laodelphax striatellus, up to a dilution of 16,000-fold by LF assay, but ELISA and PAG technique were up to 6-8 times more sensitive than LF assay. The results show PAG technique is extremely sensitive. The advantage of this technique is able to distinguish virus particles and host components in crude extracts of infected tissues. A double labelling PAG technique was applied for the simultaneous demonstration of RSV and rice grassy stunt virus (RGSV) with a similar filamentous appearance in the electron microscope. Depending on the antibodies used, RSV and RGSV in mixed suspensions were labelled specifically with PAG with gold particle diameters of 5 and 10 nm, respectively. Using this technique it was possible to distinguish and identify two viruses in the electron microscope.

R23.9

DETECTION OF ST. LOUIS ENCEPHALITIS -- JAPANESE ENCEPHALITIS VIRUS COMPLEX ANTIGENS BY CAPTURE ENZYME IMMUNOASSAY

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X.Y. Han, Z.Y. Xu. Department of Epidemiology, Shanghai Medical University, Shanghai, China.

A capture enzyme immunoassay (EIA) detects antigens of the following viruses in the St. Louis encephalitis (SLE)-Japanese encephalitis (JE) virus complex: SLE, JE, Murray Valley encephalitis (MVE), West Nile (WN), Kunjin, and Alfuy. Viral antigens are captured with 6B4A-10, a complex reactive monoclonal antibody prepared against JE virus. The detector is a horseradish peroxidase conjugate of 6B6C-1, a flavivirus group reactive monoclonal antibody.

The assay detects 50 pcg/0.1 ml of purified viral protein of SLE, JE, MVE, and WN viruses. Field caught SLE or JE virus infected mosquitoes are detected by EIA with greater sensitivity than by viral isolation in cell culture.

The speed with which results are available (1 day) and the simplicity and economy of the procedure (<$0.10/sample) should facilitate viral surveillance in mosquitoes and in predicting epidemic SLE, JE, MVE, and WN virus infections.

R23.10

DEVELOPMENT OF ELISA KIT AND MONOCLONAL ANTIBODIES FOR DETECTING CHINESE ADULT DIARRHOEA ROTAVIRUS(ADRV).* Wang Changan, Ye Weiwei, Yang Ronjian, Ma Liren, Hung Tao. Institute of Virology, Chinese Academy of Preventive Medical Sciences, Beijing, China.

A sensitive and specific, double sandwiched ELISA was developed and the ELISA kit for diagnosis of ADRV has been in use. The diagnosis made in this laboratory was very satisfactory. A high positive rate(91%) was achieved through detecting 357 stools collected from patients with ADRV. 82 samples from non-diarrhoea and 148 from normal individuals were all negative. For comparison, 48 ADRV stools were tested parallely with ELISA, PAGE and EM turned out 100% positive for ELISA, 85.4% for PAGE and 56.26% for EM. The specificity of the kit was confirmed by showing no cross-reaction with group A rota-, polio-, reo-, hepatitis A, adeno- and ECHO viruses.

10 hybridomas secreting McAb against ADRV were made from immunizing BALB/C mice with purified ADRV. 2 of the 10 were identified to be IgG2a and used equally well as polyclonals showed by ELISA. The coincident rate(88.4%) and sensitive rate (86.2%) were also being satisfactory.

*This work was supported by CDD Programme of the WHO.

R23.11

RAPID DETECTION OF PLANT VIRUSES BY ALKALINE PHOSPHATASE AMPLIFIED ELISA. L Torrance. MAFF Harpenden Laboratory, Hatching Green, Harpenden, Herts. AL5 2BD, UK

Alkaline phosphatase amplified ELISA (amplified ELISA) differs from the conventional ELISA utilising alkaline phosphatase only in the substrate reaction. In amplified ELISA bound enzyme-labelled antibody catalyzes the conversion of NADP to NAD which is then used in a secondary enzyme-mediated cyclic reaction producing a red coloured end product. Amplified ELISA has been compared to a direct antibody sandwich ELISA for the detection of viruses such as barley yellow dwarf virus (BYDV). It was found to increase sensitivity of virus detection sufficiently to allow very short incubation periods of sap and enzyme conjugate providing fast diagnostic tests. And can be used to detect BYDV in individual aphids.

R23.12

THE ANTIBODY RESPONSE TO GLYCOPROTEIN I OF AUJESZKY'S DISEASE VIRUS AS A PROBE TO DISTINGUISH INFECTED FROM VACCINATED PIGS
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Aujeszky's disease (AD;pseudorabies) is caused by a herpesvirus. It is one of the most important diseases affecting today's pig industry, worldwide. In most countries vaccination is widely practised. Consequently, for the purpose of eradicating AD, a serological assay is required to distinguish vaccinated from infected pigs. Vaccines that carry a deletion in the unique short region of the genome and as a result do not express glycoprotein I (gI) have been used for years.

We have developed a blocking ELISA (BELISA) that specifically detects antibody to gI. The BELISA is based on the use of 2 different monoclonal antibodies directed against gI. Thus, pigs vaccinated with "gI-negative" vaccines developed no antibody to gI, until they were challenge-exposed intranasally or by contact to field strains of AD virus. Field strains from Europe, Asia and America all induced gI-antibody. The gI-antibody was first detected about 2 weeks post infection and persisted at a constant level for at least 8 months.

The use of the new BELISA in conjunction with "gI-negative" vaccines opens perspectives for the implementation of combined vaccination-eradication programmes for AD.

R23.13

SEROLOGICAL VARIANTS OF MEASLES VIRUS BY HAEMAGGLUTINATION INHIBITION AND PLAQUE NEUTRALIZATION TESTS

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Abstract

Antisera raised in monkey Erythocebus patas 2 weeks post-inoculation with Wild and Schwarz measles virus strains were compared by haemagglutination inhibition and plaque neutralization tests. The HAI titres revealed significant differences by Mann-Whitney statistical analysis at a = 0.05. The plaque neutralization tests showed marked differentiation at the 50% end-point. The implication of this findings is highlighted in relation to immunization.

R23.14

THE DEVELPOMENT OF THE IgM RESPONSE IN VARICELLA AND ZOSTER EXAMINED BY IMMUNOBLOTTING. D.R.Harper, H.O.Kangro, R.B.Heath. Department of Virology, Medical College of St. Bartholomew's Hospital, London, United Kingdom.

The development of the IgM response in immunocompetent adult patients with varicella or zoster was examined by immunoblotting. MRC5 cells infected with an isolate of VZV were used as the source of antigen. Between 5 and 8 sequential sera were taken from 11 patients up to 730 days after the onset of varicella and from 12 patients up to 390 days after the onset of zoster. A total of 140 sera were tested.

All patients exhibited a detectable response, with up to 25 bands being detected, ranging from 25 to 285kDa. Sera were reacted with uninfected cellular material as a control, and any bands which were detected with control antigen were discounted. The reactivity of sera from zoster patients was greater than that of sera from varicella patients, which was unexpected. Strong virus-specific bands were detected for seven or more individuals at 34, 35, 37, 38, 30, 62 & 285kDa, and for at most three individuals at 58, 78, 82, 108, 132, 170, 174 & 182kDa. The most evident response was to a group of bands in the 34-40kDa range, and this was more marked with sera from zoster patients. Bands exhibiting the molecular weights expected for the viral glycoproteins were observed, but did not represent the major immunoreactive species, in contrast to the results obtained for IgG.

R23.15

DETECTION OF ANTIBODIES TO HIV BY WB AND IFA IN PATIENTS AND AIDS SUSPECTES. T. Gotlieb-Stematsky, A. Vonsover, R. Alexander, F. Mileguir. The Central Virology Laboratory, Ch. Sheba Medical Center, Tel-Hashomer and Sackler School of Medicine, Tel-Aviv University, Ramat-Aviv, Israel.

Determination of antibodies (abs) to human immune-deficiency virus (HIV) is based on Elisa and confirmation by Western blot (WB). Indirect immunofluorescence assay (IFA) employing Hut 78-HIV infected cells was used for detection of abs in 198 Elisa positive sera obtained from 16 AIDS patients, 48 blood donors, 59 haemophiliacs, 55 homosexuals and 20 drug abusers. 179 sera were positive by WB (du Pont, U.S.A.), reacting at least with P24 and GP41 and by IFA (titers 1:10-1:160). 19 sera (9.6%) were discordant: 11 were IFA positive and WB negative, 8 were IFA negative and reacted in WB only with P24 and/or P51/53. Discordant results were mainly observed among blood donors and WB P24 reactivity seemed to be false.

Two consecutive sera obtained from an AIDS suspect were IFA positive. However, the first serum was WB negative, while the second turned positive one month later. Follow up of IFA positive and WB negative subjectes is recommended.

R23.16

ATTEMPTS TO DETECT CYTOMEGALOVIRUS ANTIGEN IN URINE BY ENZYME IMMUNOASSAY.
P.D. Griffiths, J. McKeating, J.E. Grundy, Department of Virology, Royal Free Hospital School of Medicine, London NW3 2PF, England.

Experiments with cell culture grown CMV strain AD169 showed that monoclonal antibodies were able to capture CMV antigen and so form the basis of an enzyme immunoassay for detection of this virus. However, when clinical urine samples known to contain CMV were tested, no positive signals were obtained. Experiments showed that positive reactions could be produced if the same urine samples were held at 4°C for 2-3 weeks so that an inhibitory substance was destroyed. This inhibitor was shown to be a host protein β_2-microglobulin (β_2m). Experiments showed that the inhibitor did not affect the capture antibody but bound directly to the virus itself. Studies of CMV purified directly from clinical urines showed, by means of western blotting using monoclonal antibodies against β_2 m, that the virus is coated with this host protein. Experiments with cell culture grown CMV showed that the virus acquires radiolabelled β_2 m after release from cells.

The finding of this inhibitory host protein was entirely unexpected and the implications for the ability to detect CMV by enzyme immunoassay will be discussed.

R23.17

THE USE OF IMMUNOGOLD LABELLING FOR EM DETECTION OF VIRAL ANTIGENS IN FLUID SPECIMENS. F. Doane, N. Anderson, J. Hopley. Department of Microbiology, Faculty of Medicine, University of Toronto, Toronto, Ontario. Canada. M5S 1A8.

Immunoelectron microscopy (IEM) is one of the most sensitive of viral immunoassays. The sensitivity and visual recording of IEM is greatly enhanced by the incorporation into the test of the label colloidal gold conjugated to protein A. It has been the objective of this study to develop an immunogold method that could be used for detecting viral antigens in clinical specimens of the type routinely received in a diagnostic laboratory. Preliminary stages in the development of a protein A-gold IEM method were carried out on rotaviruses and picornaviruses in cell culture lysates. Subsequently the method was applied to clinical specimens. Comparative tests were run in parallel with other EM detection methods, including direct EM examination, Airfuge ultracentrifugation, and standard IEM. The protein A-gold IEM method was found to be more sensitive than all other methods tested, and was especially impressive in the detection of small enteric viruses.

EM methods such as these are not suited to large-scale screening of specimens; however, for selected specimens they offer a highly sensitive procedure for the detection of viral antigen. They can also be used for EM detection of low levels of antiviral antibody.

R23.18

MONOCLONAL ANTIBODIES RAISED AGAINST NEWCASTLE DISEASE VIRUS GLYCOPROTEINS. M. Nesbit & A.C.R. Samson, Department of Genetics, University of Newcastle upon Tyne, Newcastle upon Tyne NE1 7RU, England, U.K.

Three month old Balb/C female mice were immunized with purified egg-grown Newcastle disease virions (NDV). Three immunizations were carried out, the initial one in Freund's Complete Adjuvant. Hybrids produced from a fusion between disrupted whole spleen and NS1 myeloma cells were plated onto 96 well tissue culture plates. After HAT selection, tissue culture medium from the wells was screened on ELISA plates coated with virions. Using this approach three independent monoclonal antibody (Mab) producing hybrids have been isolated. All three Mabs recognise the haemagglutinin/neuraminidase (HN) protein of Newcastle disease virus (strain Beaudette C).

An alternative method of screening hybrids using NDV infected MDBK cells and the indirect peroxidase technique, (Russell et al, J. Immunol. Methods, 61: 165-170 (1983)) is now in progress, and the characteristics of Mabs obtained by this method will be compared to the others.

R23.19

Universal Rapid Micro-Neutralizing Antibody Assay (URNAA) for Influenza Virus using an EIA Format and Monoclonal Antibodies. M. W. Harmon, P. Rota, G. Burgess, and A. P. Kendal. Influenza Br. DVD, CID, Centers for Disease Control, Atlanta, GA. 30333.

The URNAA was performed using sera diluted in microtiter wells. Virus (25-100 TCID-50) was added and reacted for 1 hr, and after addition of MDCK cells (2 x 10 5 /well), plates were incubated at 37 C for 17 hr. Virus replication was detected in the wells by a universal EIA using monoclonal antibodies to influenza A or B NP antigens. The URNAA seroconversion rate for young adults who received the standard trivalent vaccine was equivalent to the HI for the H1N1 (A/Brazil) and H3N2 (A/Bangkok) components, and for influenza B (B/Singapore), was as sensitive as the HI using ether-treated antigen. The URNAA was also compared with a previously developed microneut (J. Clin. Micro. 12:426, 1980) and results for influenza A (H3N2) and B were equivalent, although URNAA was slightly less sensitive for influenza A (H1N1). Sera from humans, chickens, ferrets, mice, and rabbits could be used without treatment. The URNAA is simple, reproducible, provides results in less than one-fifth the time of previous influenza tests, and makes use of monoclonal antibody reagents that can be made widely available.

R23.20

THE USE OF BIOTINYLATED DNA IN SITU HYBRIDIZATION AND AN AVIDIN-BIOTIN IMMUNOPEROXIDASE TECHNIQUE FOR DETECTION OF LYMPHOTROPIC HERPESVIRUS INFECTION. G.D. Hsiung[1,2] T. Brady[1], B. Sun[1] Z. Nagy[1] J.J. Leary[1] Virology Lab.,[1] VA Med. Cntr., West Haven, CT., Yale Univ. School of Med.[2] New Haven, CT and Beckman Inst. Inc.[3] Brea, CA. U.S.A.

In order to develop a sensitive system for the detection of lymphotropic herpesvirus infection, biotinylated DNA in situ hybridization and an avidin-biotin immunoperoxidase (ABC-IP) technique were compared with cytopathology for the recognition of guinea pig lymphotropic herpesvirus (GPHLV) infection in cultured cells and in infected guinea pigs. DNA probes consisting of whole viral genome or cloned fragments were utilized. Rabbit anti-GPHLV sera were absorbed with guinea pig embryo cells and served as primary antisera. At an input multiplicity of ≥ 0.4, virus induced inclusions and cytopathic effect were not observed in cultured cells until 20 hrs pi, whereas viral antigen and viral DNA were first detected 12 hr pi. When tissue sections obtained from acutely infected guinea pigs were examined, viral antigens alone were found in lung tissues while cytopathology was not evident. These results suggest that both viral antigen and viral DNA detection methods could shorten the time to recognition of GPHLV infection in vitro while antigen detection by the ABC-IP method appears to be more sensitive than DNA detection by biotinylated DNA probes for the diagnosis of GPHLV infection in vivo.

R23.21

ANALYSIS OF LETTUCE NECROTIC YELLOWS VIRUS STRUCTURAL PROTEINS WITH MONOCLONAL ANTIBODIES. R.G. Dietzgen, R.I.B. Francki. Waite Agricultural Research Institute, Department of Plant Pathology, University of Adelaide, Glen Osmond, South Australia 5064, Australia.

It has been reported that lettuce necrotic yellows virus (LNYV) has five structural proteins L,G,N,NS and M, like many other rhabdoviruses. When proteins from highly purified LNYV preparations were examined in polyacrylamide gel electrophoretograms by staining with silver or Western blotting with polyclonal antibodies, three major (G,N and M) and numerous minor (up to 12) polypeptides were detected.

Monoclonal antibodies to the G and N proteins have been prepared and characterized. These monoclonal antibodies have been used to detect and differentiate glycosylated and non-glycosylated forms of the G protein. It has also been shown that a number of the minor proteins detected (up to 5) are degradation products of the N protein. The degradation products are readily detected in purified virus preparations but not in extracts from infected plants suggesting that they are produced in vitro. Hybridomas secreting antibodies to the L, NS and M proteins are being screened and when available, should be useful in further analysing the structural proteins of LNYV.

R23.22

STUDIES ON THE SEROLOGICAL DIAGNOSIS USING MICROSPHERES. H.Mizutani and H.Mizutani. Department of Virology,Kanto Teishin Hospital, Tokyo, Japan.

The purpose of this work is to develope a simple procedure for serological diagnosis using microspheres. TORAYSPHERES(Toray Industries,Inc. Tokyo,Japan) were used as microspheres. They are composed of acrylic polymer having functional groups. A/Bangkok/10/83(H1N1),B/Ibaragi/2/85, strains of influenza viruses,HF strains of Mycoplasma pneumoniae, etc. were used. Concentrated organisms were treated by ether(H.Mizutani,et al. Virology 17,210,1962) or acetone(H.Mizutani,Amer. Rev.Resp.Dis.,111,566,1975) etc. Treated antigens were fixed to activated microspheres according to a variety of procedures. The results of serological diagnosis using the sensitized microspheres were compared with that of another methods. Our results showed an advantage of using the TORAYSPHERES that it may facilitate the differential diagnosis of systemic infectious diseases.

R23.23

EPITOPE ANALYSIS OF POTYVIRUS COAT PROTEINS USING MONOCLONAL ANTIBODIES. Ramon L. Jordan and John Hammond. USDA-ARS, BARC-West, FNCL, Beltsville, Md. 20705 USA.

Forty-three monoclonal antibodies (McAbs) were generated using a mixture of serologically related and unrelated potyviruses as immunogen and screening antigens. Twenty of the McAbs react only with one or more of the six bean yellow mosaic virus (BYMV) isolates tested. The remaining 23 McAbs react with a BYMV isolate and with at least one of the other 30 potyvirus isolates tested.

Approximately 27 different potyvirus coat protein epitopes can be defined with the 43 McAbs based on antigen specificity using the 30 different potyvirus isolates. McAb reactivities indicate the presence of conserved antigenic determinants common to members of the potyvirus group previously characterized as serologically distinct or unrelated. McAbs reacting with apparently conserved epitopes are specific to antigenic sites not found on the surface of intact virions. At least one McAb reacts with the trypsin-cleaved N-terminal peptide of BYMV coat protein. McAb reactivities in various ELISA, immuno-blot, and western-blot analyses with potyvirus coat proteins, peptide fragments and specific fusion proteins expressed in bacteria containing cloned inserts of the viral coat protein gene will be presented.

R23.24

UTILIZATION OF THE HIGHLY PERMISSIVE C3 CELL LINE IN RAPID SERUM NEUTRALIZATION ASSAYS. W. Edward Robinson, David C. Montefiori and William M. Mitchell, Vanderbilt University, Nashville, Tennessee, USA.

The HTLV-II transformed cell line C3 is highly permissive to HIV infection in vitro. Even in the presence of low MOI (0.01-0.1), this cell line exhibits rapid expression of viral antigens in 4-6 days with extensive cell lysis as early as 7-9 days post virus adsorption. Utilizing the C3 cell line, a rapid neutralizing antibody screen is now described. Demonstrated are results derived from patient sera obtained during the course of antiviral drug therapy. Using either an increase in time to cytolysis, reduction in reverse transcriptase assay values, or reduction in percent indirect immunofluorescence as the criteria for neutralizing of virus, it is possible to clearly demonstrate significant differences in the anti-HIV qualities of different patient sera. Some patients show significant neutralizing ability with either percent fluorescent cells or reverse transcriptase levels less than thirty percent of HIV infected control. Other patients show virtually no ability to neutralize HIV with RT assay values or percent fluorescence eighty percent of control values. Therefore, utilizing standard neutralizing antibody titer techniques but incorporating the C3 cell line, it is possible to completely evaluate patient serum in seven days. The rapidity with which this cell line expresses viral antigens makes the C3 the cell line of choice in serum testing especially when large numbers of sera must be evaluated.

R23.25

ETIOLOGICAL AND EPIDEMIOLOGICAL STUDIES OF HEMORRHAGIC FEVER WITH RENAL SYNDROME IN SICHUAN ENDEMIC AREA

Li-Li Chen, Yun-Ju xie, Shang-Zhi Chen, Guo-Ying Zheng, Wen-Ting Tao and Guo-Fang Tao

Department of Viral Disease Control, Health and Antiepidemic Centre of Sichuan, Chengdu, People's Republic of China

It is the first time to isolate 10 strains of Hantaan viruses from Rattus norvegicus, Rattus flavipetus, Anourosorex squamipes, crocidura russula and HFRS patients in Sichuan endemic area. Serological identification of these strains was made by IFAT and ELISA techniques. Preliminary antigenicity analysis of the viruses was carried out with monoclonal antibodies of Hantaan virus. The results showed that all the strains tested are antigenically closely related to A9 and Hantaan virus 76-118 strains that belong to Apodemus type. It was not reported before in our country that the Apodemus type virus was isolated from house rats. The epidemiological significance of the results have briefly discussed in this paper.

R23.26

AN UNIQUE HERPESVIRUS FROM A PATIENT WITH CHRONIC ACTIVE EPSTEIN-BARR VIRUS (CAEBV) INFECTION. M. Okano, G. Thiele, J. Davis, W. Nauseef*, D. Purtilo. Dept. Pathology/Microbiology, Univ. Nebraska, Omaha, NE 68105, Dept. Medicine*, Univ. Iowa Hospitals, Iowa City, Iowa.

A herpesvirus was isolated from peripheral blood lymphocytes (PBL) and throat washings from a 24-year-old man who had infectious mononucleosis followed by CAEBV. A lymphohistiocytic infiltrate, erythrophagocytosis, and transformed lymphoblasts were seen in tissues at autopsy. Antibody titers to viral capsid and early antigens were 1:40,960 and 1:2560, respectively. EBV genome was detected in his spleen by Southern blot hybridization. Oligoclonal B-cell populations were detected in his spleen with J_H hybridization. Attempts to immortalize cord lymphocytes by infecting with throat washings, cell-free supernatants or by co-culturing with peripheral blood leukocytes (PBL) were unsuccessful due to a marked cytopathic effect (CPE). The CPE was accelerated (within 2-4 days) when TPA or n-butyric acid were added. Cultured cells and supernatant contained particles with herpesvirus group morphology. Immunofluorescent staining of infected cells using monoclonal antibodies and hyperimmune serum excluded all herpesviruses. In contrast, the patient's serum reacted strongly with infected cells as did serum seropositive for human B lymphotropic virus. This novel herpesvirus may be an etiologic agent of the CAEBV syndrome and other lymphoproliferative diseases. Supported by PHS CA30196.

R23.27

EPSTEIN-BARR VIRUS NUCLEAR ANTIGENS IN BURKITT'S LYMPHOMA CELL LINES. T.B. Sculley[1], D.G. Sculley[1], J.H. Pope[1], G.W. Bornkamm[2], N. Mueller-Lantzsch[2], G.M. Lenoir[3] and A.B. Rickinson[4]. 1. Queensland Institute of Medical Research, Bramston Tce., Herston, Brisbane, Australia 4006. 2. Institut fur Virologie, Zentrum fur Hygiene der Universitat Freiburg, Hermann-Herderstr. 11, 7800 Freiburg, West Germany. 3. International Agency for Research on Cancer, Lyon, France. 4. Department of Cancer Studies, University of Birmingham Medical School, Birmingham, B15 2TJ, U.K.

Epstein-Barr virus (EBV) has previously been classified into two different types according to the organization of the EBNA2 gene region. Type A virus hybridizes with probes from B95-8 or M-ABA viruses, and the B type virus with probes from the Jijoye virus strain. The substituted region in EBV type B codes for a different, but related EBNA2 antigen named EBNA2B as opposed to EBNA2A. In this study Burkitt's Lymphoma (BL) cell lines, previously typed according to the EBV viral genomes they carry, have been examined by immunoblotting for the expression of both EBNA1 and EBNA2 antigens. EBNA2A was identified in all lines carrying A type viral genomes, but was not observed in any of the lines harbouring B type virus, though BL lines containing B type virus did not always express the EBNA2B antigen. The association of B type EBV with BL is discussed.

R23.28

RAPID DETECTION OF INFLUENZA VIRUSES WITH MONOCLONAL ANTIBODIES TO M-PROTEIN. D. Bucher, A. Mikhail, S. Popple, M. Baer and C. Whitaker. Dept. of Microbiology, Mt. Sinai School of Medicine, New York, N.Y. 10029, U.S.A.

A panel of 14 distinct hybridoma lines secreting monoclonal antibodies reactive with type A influenza virus M-protein (A/PR/8/34) has been developed. Monoclonal antibody titers (ELISA) range from 0.2-5.8 X 10^6 vs. M-protein and $\leq$0.3 X 10^3 to 4.3X 10^5 vs. X-53a (H1N1) influenza virus. Three distinct epitopes are recognized as determined by competitive inhibition assay. Isotypes represented are primarily IgG_1 (11 lines) with single lines secreting IgM, IgG_{2a} and IgG_{2b}. Specificity for M-protein was verified by Western blot analysis. Virus could be detected to a level of 10 ng (twofold level of absorbance above background) utilizing the monoclonal antibodies as 'capture' antibodies. Combinations of antibodies recognizing different epitopes enhanced sensitivity. Purified antibodies from rabbit hyperimmune M-protein antisera served as 'sandwich' antibodies. Goat anti-rabbit antibodies conjugated with alkaline phosphatase provided enzyme detection. Triton X-100 was added to a concentration of 0.09% to expose M-protein in the virus for 'capture'. The monoclonal antibodies detected H3N2 and H1N1 human influenza virus isolates following amplification. The monoclonal antibodies also permitted immunofluorescence visualization of M-protein in infected cells.

R23.29

RAPID DETECTION OF CYTOMEGALOVIRUS IN BRONCHO-ALVEOLAR LAVAGE SAMPLES: IMMUNOFLUORESCENCE STAINING OF LAVAGE CELLS, SHORT TERM AND STANDARD CULTURE TECHNIC. M.Harzic, Françoise Brun-Vezinet; Laboratoire de Virologie Hopital Claude Bernard, Paris - France.

94 broncho-alveolar lavages (BAL) were performed in 77 immunocompromised patients (pts): 73 pts were HIV-1 seropositive with AIDS or severe ARC, 4 were HIV-1 seronegative (1 pt has had a renal allograft, 2 had malignancies and one was aplasic).

BAL cytospin preparations were fixed in cold acetone and stained by indirect immunofluorescence technique (IF) with a monoclonal antibody to an immediate early antigen of CMV (E13*). In standard culture MRC5 cells were inoculated with a filtrate of the BAL and examined for typical CPE. 63 samples were culture positive; in 8 samples, IF on BAL cells was not interpretable; 60% of the 55 other where IF positive. IF was never positive in culture negative samples.

We also performed a short term culture technique in 50 samples: MRC5 cells were inoculated with a filtrate of the BAL, centrifuged at 37°C at 3000 g for 1 H and stained after 48 H using a immunoperoxydase assay with E13. Results in short term culture and standard culture concord.

We are analyzing these results comparatively to the cytological and clinical data.

*E13 was a gift from Pr.Perol, Hopital Saint Louis.

R23.30

COMPARISON WITH FOUR TESTS IN THE EBV ANTIBODIES DETECTION. G.Alegente,F.Mugnaini,B.Marchi. Lab.Bacteriology,S.Maria Hospital,Siena,ITALY.

Epstein-Barr virus EBV,is the most recently described member of the human herpesvirus group.Infection with EBV is common world-wilde in distribution and largely subclinical in early childhood.EBV has been established as the etiologic agent of hete-rophile-positive infectious mononucleosis,which oc-curs most frequently in late adolescence or early adulthood.In the present investigation we have utilized four tests:IgG and IgM ELISA (VIRION),IFA(ELE-CTRO-NUCLEONICS),MONOSPOT (ORTHO DIAGNOSTIC SYSTEM and CF (VIRION).

A total of 340 serum specimens from hospitalized patients were tested,using the CF test as a referee Sensivity and specifity of the ELISA were calculated to be 92,4 and 93,9% respectively;sensivity and specifity of IFA were 99,1 and 99,5%respectively. The MONOSPOT test was compared with CF test and there was 89,6% agreement.

R23.31

PRENATAL DIAGNOSIS OF AND FETAL IMMUNE RESPONSE TO CONGENITAL VARICELLA INFECTION RESULTING IN NEUROLOGIC EMBRYOPATHY. G. Cuthbertson, C. Weiner, C. Grose. Departments of Pediatrics and Obstetrics, Faculty of Pediatric Infectious Diseases, University of Iowa, Iowa City, Iowa, U.S.A.

Detailed immunologic studies were carried out on sera from a case of congenital varicella virus (VZV) infection with severe central nervous system (CNS) malformations. CNS embryopathy was evaluated by computed tomography and magnetic resonance imaging. Sera were obtained by intrauterine cord canulation at 34 weeks gestation (10 days before birth) from a primagravida with history of a VZV infection in the second trimester at 20 weeks gestation.

Using fluorescent antibody to membrane antigen (FAMA) method, we demonstrated VZV specific IgM titers of 1:16 in the fetal blood. After separation of IgM from IgG by sucrose gradient ultracentrifugation, the response of specific IgM to individual VZV surface glycoproteins was studied by both radioimmune precipitation and gel electrophoresis techniques. The fetal immunoprecipitation profile showed production of antibody to the 3 major VZV surface glycoproteins: gpIII(118), gpI(98), and gpII(66). After birth the infant's production of IgM fell sharply, as demonstrated by a 4-fold fall in the immunoprecipitation % recoveries.

These results allowed us to make the prenatal diagnosis of intrauterine varicella infection and show, for the first time, a fetal IgM response to individual VZV glycoproteins.

R23.32

RAPID DIAGNOSIS OF INFLUENZA A AND B BY 24 HOUR FLUORESCENT FOCUS ASSAY. C.E. STOKES[1] AND J.M. BERNSTEIN[1,2], Res. Svc.[1], VAMC, OH and Wright St. U. Sch. Med.[2], Dayton, OH, USA.

Immunofluorescent (IF) antibody probes have been shown to be useful for the rapid detection of viral antigens in infected shell vial cell culture monolayers. Multiwell chamber slides offer an alternative to individual shell vials. We have developed a chamber slide-fluorescent focus assay (CS-FFA) using monoclonal antibodies directed against non-subtype specific antigens of influenza (INF) to identify INF A or B within 24 hours of specimen receipt. This was compared to both culture tube isolation (CT) and IF of exfoliated nasopharyngeal cells (NW-IF) for the diagnosis of INF.

38 nasal washes were obtained from 13 adult patients (age 44 ± 9 years) with symptoms of INF. 16 specimens were positive for hemadsorbing agents by standard CT technique (13 INF A and 3 INF B). The time to isolate INF by CT was 7 ± 4 days. Of the 16 INF isolates, 10/13 (76%) INF A isolates and 3/3 (100%) INF B isolates were identified by CS-FFA 16-24 hours after inoculation. Sensitivity and specificity of CS-FFA were 81% and 91% with respect to CT. An additional 24 hour incubation did not increase the positive yield. NW-IF identified 5/13 INF A isolates (38%) and 1/3 INF B isolates (33%). We conclude that CS-FFA is a rapid and sensitive technique for the diagnosis of INF A and B in adults. CS-FFA may be preferable to shell vial assays since multiwell CS-FFA requires less technical manipulation.

R23.33

CLINICAL FEATURES OF HTLV-I RELATED P-40 POSITIVE PATIENTS. I. HONDA, A.ISHIMORI, & S. WATANABE. Department of Clinical and Laboratory Medicine, Tohoku University School of Medicine, Sendai, Japan.

The mechanism of malignant transformation by HTLV-I is still unknown, but recently a new gene, termed "x" was identified in HTLV-I which has been hypothesized to be the transforming gene of HTLV because of its conservation within the HTLV class of retroviruses.

We investigated the significance of Px gene derived P-40 as an etiological marker of viral replication and the relationship of clinical manifestations. By the Western blot method anti P-40 bands were noted in 3 out of 7 sera of typical ATL, in 1 serum of HTLV-I associated myelopathy, furthermore noted in 6 sera/ 5 cases among 40 antibody positive sera for HTLV-I/ 28 cases (out of 2430 specimens which were corrected from inpatients and outpatients outside the endemic area of ATL in Japan); 2 cases of chronic bronchobronchiolitis with persistent airway infections with P.aeruginosa besides the skin lesions, 2 cases of post blood transfusion states because of severe anemia with myoma uteri in 1 case and hemorrhagic gastric ulcer in 1 case, another case was Crohn's disease. All cases showed 1 - 2% atypical lymphocytes with lymphocytopenia in peripheral blood, suggestive smoldering leukemia.

R23.34

ANTIGEN DETECTION IN DIAGNOSIS OF CRIMEAN-CONGO HEMORRHAGIC FEVER. A.J. Shepherd and R. Swanepoel. National Institute for Virology, Sandringham, 2131, Republic of South Africa.

Enzyme-linked immunosorbant assay (ELISA) and reversed passive hemagglutination (RPHA) tests were evaluated for rapid detection of Crimean-Congo hemorrhagic fever (CCHF) virus antigens. Both RPHA and ELISA detected CCHF antigen in the brains of infant mice 2 to 3 days after infection; several days before the animals sickened and died. Antigen was also detected after 1 to 2 days in infected cell culture extracts and after 2 to 4 days in culture supernatant fluids. Both tests detected CCHF antigen at threshold values of approximately 10^3 tissue culture infective doses per ml of infectivity and were more sensitive than complement fixation, immunodiffusion or immunofluorescence. In a comparative study on specimens from CCHF patients, virus was isolated from 38/49 sera and from 23/28 patients. Antigen was detected in 20/49 sera (15/28 patients) by RPHA and in 29/49 sera (18/28 patients) by ELISA. Antigenemia was detected more frequently in fatal cases (9/11) than in non-fatal cases (9/17). The results suggest that RPHA and ELISA may be of use in rapid diagnosis of CCHF infection, particularly in severe cases where the danger of nosocomial spread is greatest.

R23.35

DIAGNOSIS OF BLUETONGUE INFECTION USING A GENETICALLY ENGINEERED BLUETONGUE ANTIGEN. Polly Roy[1], S. Inumaru[2], & J. Pearson[3]. [1]University of Alabama, Birmingham, AL 35249 U.S.A., [2]Institute of Virology, Mansfield Road, Oxford U.K. and [3]NVSL, Ames, Iowa 50010, U.S.A.

Bluetongue is an arthropod-borne disease of sheep and cattle caused by a double-stranded RNA virus. The genome that containst 10 RNA species, is enclosed by a double-capsid, of seven polypeptides. The two inner capsid proteins, VP3 and VP7 are believed to be group-specific antigens of 24 bluetongue serotypes. DNA representing RNA segment 3 of bluetongue virus (BTV) serotype 17 corresponding to the gene that codes for VP3 has been manipulated to synthesize the VP3 antigen, in insect cells Spodoptera frugiperda. The authenticity of the VP3 protein was demonstrated by raising antibodies in rabbits and mice to this protein and then used it in immunoprecipitation studies. In order to determine if VP3 protein produced in insect cells could react with homologous or heterologous BTV antisera, the VP3 antigen was absorbed to the microtitre plates followed by incubation with different BTV-antisera. The antigen-antibody complexes were then detected by alkaline phosphatase-protein A method. All BTV antisera (1:1000 dilution) reacted with the antigen significantly high level in comparison to the control non-immune sera or normal rabbit serum.

R23.36

RAPID DIAGNOSIS OF RESPIRATORY SYNCYTIAL VIRUS INFECTIONS. D.C. Halstead, Departments of Microbiology and Immunology, HealthEast Laboratories, Allentown, PA, U.S.A.

Respiratory Syncytial virus (RSV) is a major cause of respiratory disease in children less than 2 years old. With the advent of ribavirin therapy and the potential for nosocomial spread, a need for rapid RSV diagnosis triggered an evaluation of 125 specimens tested in parallel by an enzyme immunoassay (EIA) and a direct fluorescent monoclonal antibody test (DFA) for RSV antigen in nasal aspirates. 67/125 were also inoculated into HEp-2 cells and observed for typical cytopathic effect. In contrast to previous reports, nasal secretions were examined by DFA without prior washing and centrifugation in order to expedite results and decrease hands-on time in processing. 58/125 specimens were confirmed RSV positive by culture or by at least two of the three methods. Using this criteria, 56 specimens were considered true positive by DFA and 56 by EIA. The sensitivity, specificity and predictive values positive and negative of both DFA and EIA were the same: 97% and 97.5% and 97% and 97.5%, respectively. Elimination of a laborious washing procedure and implementation of a direct monoclonal RSV reagent appears to be an acceptable screen for RSV positive specimens in laboratories with an experienced fluorescent microscopist.

R23.37

SCANNING/TRANSMISSION IMMUNE ELECTRON MICROSCOPY AS AN AID IN DETECTION OF HIV ANTIGENS IN HUMAN PERIPHERAL BLOOD LYMPHOCYTES. R.Méndez,M.I.Herrera,I.Santa María,R.de Andres,J.M.Varela,C.Casado ,M.Domínguez,Centro Nacional de Microbiologia,Virología e Inmunología Sanitarias.Instituto de Salud Carlos III. Majadahonda(Madrid), Spain.

Scanning immune electron microscopy(SIEM) might prove to be helpful in the search for HIV antigens during replication and in rapid AIDS diagnosis early in infection. It provides a panoramic overview of the morphological surface changes of HIV infected cells by secondary electrons(SE) imaging combined with the precise identification by backscattered electrons(BSE) detection of immunogold labelled viral antigens at the cell surface. When scanning-transmision electron microscopy(STEM) is used,the sensitivity of the technique increases, as viral antigens can be detected even below the cell surface, before budding occurs.

Routine SEM studies of HIV infected lymphocytes reveal the presence of characteristic giant cells of "spongy" surface appearance that have been also observed in blood from 13 HIV seropositive patients. In four of them other unusual spherical structures were also found. They could correspond with a process of amplification of a viral gene and could indicate virus production at low level. To characterize more precisely the relationship between - the morphological changes and viral infection,HIV infected cell cultures - have been prepared for SIEM by means of a two step labelling procedure,with monoclonal anti-p17 and anti-gp41 antibodies as primary antibodies and 40nm gold-linked goat antimouse IgG as secondary antibodies. Paired SE and BSE micrographs have been taken showing that while normal lymphocytes give no signal,"spongy" cells contain gold particles as an indicator of the ongoing HIV replication process.

R23.38

TIME-RESOLVED FLUOROIMMUNOASSAY OF FASTIDIOUS ENTERIC ADENOVIRUS SPECIES 40 AND 41 IN STOOL SPECIMENS. M.Brown[1], N.Singh-Naz[2],P.J. MIDDLETON[2]. Department of Virology, The Hospital for Sick Children, Toronto, Canada[1] and Department of Infectious Disease, Children's Hospital, National Medical Center, Washington,D.C.,U.S.A.[2]

A time-resolved fluoroimmunoassay (TR-FIA) has been developed for the direct assay of Ad40 and Ad41 in stool specimens. The assay uses a polyclonal antiserum to Ad40 as a capture antibody and a monoclonal antibody specific for Ad40 and Ad41 as the primary detecting antibody. This monoclonal antibody was produced against purified Ad40 virions and recognizes both Ad40 and Ad41 but does not cross-react with any other adenovirus species. Binding of the monoclonal antibody is detected using labelled anti-murine antiserum. There appears to be a good correlation between the results obtained by TR-FIA and those obtained by restriction analysis of viral DNA from high salt extracts of infected 293 cells. Because TR-FIA does not require isolation of the virus in cell culture, it is a rapid technique, with results available in hours as opposed to days for DNA restriction analysis and weeks for conventional serotyping.

R23.39

SUBTYPE CROSS-REACTIVE MONOCLONAL ANTIBODIES RAISED AGAINST INFLUENZA VIRUS HEMAGGLUTININ SUBUNITS. J. A. Melero and A.Sánchez Fauquier. Centro nacional de Microbiología. Majadahonda. Madrid. Spain.

Influenza virus hemagglutinin subunits (HA1 and HA2) have been purified by preparative electrophoresis under denaturing conditions. Mice immunized with each subunit were used for the preparation of monoclonal antibodies. Most of these could recognize not only the immunizing subunit but also native hemagglutinin. Most anti-HA1 and -HA2 antibodies reacted exclusively with viruses of the immunizing subtype, when tested against a panel of reference strains.

Antibody mixtures have been worked out as subtyping reagents of type A influenza viruses. They can be used in RIA, ELISA, IFF and Western blotting techniques.

In addition, monoclonal antibodies against the matrix and nucleoprotein have been obtained which differentiate influenza virus serotypes . All these antibodies can be useful reagents for rapid influenza diagnosis.

R23.40

COMPARATIVE PERFORMANCE OF HIV ELISA TEST KITS EVALUATED ON PROBLEM SPECIMENS FROM HIGH RISK SCREENING S. Hooms, G. Shell, C. Cossen, J. Meissinger, M.S. Ascher. Serology Section, Viral and Rickettsial Disease Laboratory, California Department of Health Services, Berkeley, California 94704 USA

Sera from California public health and alternative test site laboratories are referred to the VRDL after local HIV antibody screening tests for the purpose of confirmation of positive results, for evaluation of inconsistent or discrepant Elisa tests or because of local problems with testing. From these specimen sources we identified 13 specimens which were false-negative, i.e. positive on an indirect immunofluorescent confirmatory test after a negative EIA screening result on the early Abbott kit. All 13 specimens were positive when tested by test kits from DuPont and Genetic Systems. A group of 118 Abbott false-positives showed only 4 and 1 false positives on the other kits respectively. An Abbott kit change released in July 1986 improved the specificity but false-negatives remained. A newly licensed kit from Abbott (January 1987) correctly identified all previous problem specimens. Using this improved kit and kits from DuPont, Organon-Teknika and Genetic Systems, a panel of 200 IFA positives and 100 IFA negatives was tested. All kits had qualitatively "perfect" performance on this panel with minor differences in separation of the populations. Other licensed kits gave varying degrees of sensitivity and specificity when tested on this panel.

R23.41

APPLICATION OF THE ULTRAMICROANALYTIC SYSTEM FOR VETERINARY MEDICINE DIAGNOSIS. Maritza Barrera, Julia Noda, Rocío Larramendy and J.L. Fernández-Yero. Department of Virology-Immunology, National Centre for Animal and Plant Health, Post Box 10, San José de las Lajas, La Habana, Cuba

The ultramicroanalytic system (UMAS) can determine fluorescence in 10 µL volume samples, capable of 50 determinations per minute. The aim of the present paper was to apply UMAS for the detection of antibodies against different virus and rickettsias by means of the ELISA technique with the consequent economy in terms of time and reagents.

Infectious bovine rhinotracheitis (IBR) virus, bovine leukosis virus, and Anaplasma marginale semipurified antigens were prepared. Optimum antigen concentration for each organism was determined, as well as trialed serum dilution, and incubation time and temperature. The sensibility and specificity of UMAS in said conditions disclosed satisfactory results in relation to other techniques used for the detection of these pathogens.

A computer programme for UMAS data analysis concerning IBR virus was elaborated that could be used for all other in national surveys for diagnosis and disease control programmes is highlighted.

R23.42

RAPID DIAGNOSIS OF CYTOMEGALOVIRUS (CMV) INFECTION IN THE URINARY SEDIMENT BY A MONOCLONAL ANTIBODY METHOD. A. NOVILLO, J. CELLO, C. SOSA, M. C. FREIRE. División of Viral Diagnosis,Instituto Nacional de Microbiología Carlos G. Malbrán,Buenos Aires, Argentina.

CMV is the most common viral infection affecting the fetus or the newborn. We tried to find a rapid method for diagnosing symptomatic infected infants(under 1 month of age,using monoclonal antibodies to CMV in an indirect immunofluorescence (IFI) of the urinary sediment.

Twenty urine specimens were prepared for Electron Microscopy by the diffusion through agar technique; four of them were positive while eight were positive by the IFI test .

CMV was isolated from eight out of nine urine specimens inoculated in human fibroblast. Fluorescent cells in the urinary sediment were seen in seven of the eight culture positive specimens (87,5 per cent).

With the IFI test it was possible to diagnose CMV infection within two hours after collection of the sample and the identification of CMV infected cells was easy because of bright nuclear fluorescence and low background. This method could provide advantages for its rapidity, low cost and easy performance in diagnostic laboratories.

R23.43

DETECTION OF THE VIRUS-CODED 30K PROTEIN IN LEAVES INFECTED WITH TOBACCO MOSAIC VIRUS. K. Tomenius[1], D. Clapham[1], T. Meshi[2]. Department of Plant and Forest Protection, Swedish University of Agricultural Sciences, Uppsala, Sweden[1] and Department of Biophysics and Biochemistry, Faculty of Science, University of Tokyo, Tokyo, Japan[2].

The 30K protein of tobacco mosaic virus, TMV, has been proposed to function in cell-to-cell transport of virus, probably via the plasmodesmata.

In order to localize the 30K protein inside the cells immunogold cytochemistry was used on ultrathin sections of leaves mechanically inoculated with TMV at various times before fixation and embedded at low temperature (-30°C).

Antibodies against the 30K protein were raised in rabbits with a synthetic peptide corresponding to the C-terminal sequence of the 30K protein and coupled to bovine serum albumin. Affinity purified IgG to the 30K protein or antibodies to TMV were applied to sequential sections.

The 30K protein label was found mainly inside plasmodesmata; most of which were labelled 24 hours p.i., and before the appearance of appreciable quantities of virus inside plasmodesmata.

R23.44

CYTOMEGALOVIRUS IMMUNOBLOT CONTROL DURING THE POSTOPERATIVE PERIOD OF KIDNEY TRANSPLANTED PATIENTS.

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Institut für Medizinische Mikrobiologie und Klinik für Innere Medizin; Medizinische Universität zu Lübeck, 2400 Lübeck, West Germany.

Enzyme-immuno-assays (ELISA) for CMV-IgG and CMV-IgM antibodies with sera of kidney transplanted patients often don't allow sufficient interpretation of the status concerning CMV-infection. Especially high CMV-titers with IgG and negative for CMV-IgM put problems. The idea was to use the immunoblot technique to look for the development of antibodies of the IgA, IgG, and IgM class to CMV-specific antigens in sequential serum samples of kidney transplanted patients.

The results show that in contrast to a commercially used ELISA the immunoblot technique allowed to detect CMV-IgM antibodies to CMV-specific antigens - mostly to the 155 kd antigen. This effect is more pronounciated if the ELISA-CMV-IgG titers have higher values. This could indicate that low ELISA- CMV-IgG titers are more related to a silent CMV-status whereas the higher ELISA-CMV-IgG-titers without ELISA-CMV-IgM reflect a low but permanent CMV activity.

R23.45

COMPARISON OF POLYCLONAL AND MONOCLONAL ANTIBODIES FOR TYPING OF HUMAN ROTAVIRUSES BY SOLID-PHASE IMMUNE ELECTRON MICROSCOPY. G. Gerna, N. Passarani, A. Sarasini, M. Parea, B. S. Coulson.
Virus Lab , Inst. Infectious Diseases, University of Pavia, 27100 Pavia, Italy.

Using solid-phase immune electron microscopy, a comparative study between cross-absorbed polyclonal hyperimmune sera (PAbs) and neutralizing (Nt) monoclonal antibodies (MAbs) to VP7 of the 4 human rotavirus (HRV) serotypes was conducted to test sensitivity of the two panels of reagents for typing HRV strains directly in stool specimens. While specimens rich in virus particles were easily typed by both PAbs and MAbs, the former appeared much more sensitive in typing strains poorly represented in stool extracts. In addition, a few group A HRV strains did not react at all with MAbs. Pools of MAbs to different Nt epitopes of VP7 and VP3 should be employed in typing HRV strains from different geographical areas. Partially supported by WHO, contract HQ/86/075264 and by Min. Sanità, IRCCS Pol. S.Matteo, Ricerca corrente 1985.

R23.46

DETECTION OF RESPIRATORY SYNCYTIAL (RS) VIRUS AND ROTAVIRUS IN CLINICAL SAMPLES BY CHEMILUMINESCENT ENZYME-LINKED IMMUNOSORBENT ASSAY. A. Hornsleth, K. Aasen, M. Gundestrup.
Department of Clinical Virology, Faculty of Medicine, University of Copenhagen, Copenhagen, Denmark.

An apparatus of comparatively simple construction has been developed for the detection of chemiluminescence produced in immunoassays performed in microwell modules. The light emission produced by peroxydase catalysed oxidation of luminol was enhanced by paraiodophenol and was registered on photographic film in this PREC-detector. The detection limit for purified RS- and rotavirus antigens as well as titres of RS- and rotavirus-positive clinical samples examined in this detector, could be increased fourfold compared to results obtained with more conventional sensitive ELISA-techniques.

R23.47

VIRAL ETIOLOGY IN ACUTE LOWER RESPIRATORY INFECTIONS (ARI) IN CHILDREN FROM A CLOSE COMMUNITY.
G. Carballal, M. Avila, H. Rovaletti, M.C. Weissenbacher.
Department of Microbiology. Faculty of Medicine, Buenos Aires and "Noel Sbarra" Hospital, La Plata. Buenos Aires ARGENTINA.

From June 1984 to June 1986, children under five years with lower ARI living in an orfan house and coming from low income families were studied. Sixty nine % were under 1 year and 58% males. 204 nasopharyngeal aspirates (NPA) were studied by both a rapid technique - IFI- and isolation in MRC-5, Hep-2, LLC-MK2 and MDCK cells.

NPA cells were stained for RSV,adenovirus, Flu A and B and Para Flu 1 and 3. Clinical entities observed were: bronchitis 46%; pneumonias 24%; bronchiolitis 21% and bronchopneumonias 8%.

Viral etiology was obtained in 21% of cases. From these, 53% corresponded to RSV; 19% to unidentified viruses; 14% to adenovirus; 7% to Flu A; 5% to Para Flu 3 and 2% to RSV plus adenovirus. RSV peaked in winter and was found in 14% of pneumonias, 11% of bronchiolitis and bronchopneumonias and 9% of bronchitis.

R23.48

DIAGNOSIS OF RSV INFECTIONS BY THE DETECTION OF RSV-SPECIFIC IgG, IgA AND IgM. M.J.H. Maddens, P. Berbrink, J. Lindeman and W.C. v. Dijk. SSDE, Department of Pathology, Immunology and Microbiology, P.O. Box 5010, 2600 GA Delft, The Netherlands.

For serodiagnosis of respiratory syncytium virus (RSV) infections an indirect ELISA was developed. A clinical RSV isolate grown in HEp2-cells was used as antigen.

To evaluate the ELISA results of serum pairs from 27 children aged 0-7 year collected during the summer (RSV negative group) were compared with those from 29 children of same age with proven RSV infection. In the RSV negative group no significant rises of IgG and IgA nor IgM were measured. In the RSV positive group 13 paired sera showed a $\geq$ 4-fold RSV-IgG rise. In 19 patients rises of RSV-IgA was detected and in 8 patients IgM. So 86% could be diagnosed by ELISA. During the RSV season another 17 serum pairs from 0-7 year old children with clinical signs of respiratory illness but without RSV-isolation were tested. In this group significant rises of IgG were found 2x, of IgA 2x and 2x IgM was demonstrated suggesting recent RSV infection in 6 children. With complement fixation (CF) and ELISA significant antibody rises were found in 10 out of 48 serum pairs, whereas with ELISA additionally 22 rises were found.

Thus, this ELISA was specific, was 3x as sensitive as CF and can be used as a diagnostic method for RSV infections.

R23.49

ROTAVIRUS ANTIGEN DETECTION BY VELCIA

J.Grom, Z.Zeleznik, Z.Valenčak, VTOZD za veterinarstvo, Biotechn. faculty, Ljubljana, Yugoslavia

Virus enzyme-linked cell immunoassay - VELCIA was developed for the detection of rotavirus antigen in cell monolayers on microtiter plates.

Pancreatin-activated wild-type porcine rotavirus can be detected within 24h on cell culture MA-104 using VELCIA. Immunoperoxidase labelling of microplates previously used in VELCIA has given comparable results with VELCIA and also allowed surveillance of antigen reaction on the cell sheet. Results of VELCIA were also compared with ELISA and immunoelectrophoresis. The results of 39 samples tested by the three methods did not differ greatly in the total number of positive, although individual fecal samples will vary a lot in their degree of positivity. A good correlation between the tests was found.

With VELCIA we were able to titrate OSU strain of porcine rotavirus in dilutions higher than 10^{-6} (50 µl/well) and also directly from the feces wild-type porcine rotavirus in dilutions higher than 10^{-3} (50 µl/well). This is not possible using the plaque technique. By the use of the spectrophotometer reliable results are obtained in 48 h.

R23.50

DIAGNOSIS OF HIV INFECTION: UTILITY OF IMMUNOASSAYS FOR DETECTION OF ANTIBODIES TO VIRAL STRUCTURAL PROTEINS. G. J. Dawson, S. G. Devare, D. A. Paul, M. D. Leuther, R. A. Gutierrez, R. G. Allen, J. S. Webber, J. M. Casey, J. C. Hunt, E. Chan, L. Paul, J. S. Heller, R. H. Decker, Abbott Laboratories, North Chicago, IL U.S.A.

Antibody response to the transmembrane protein of HIV (gp41) and to the HIV core protein (p24) were determined using two highly specific and sensitive immunoassay systems. The first system utilized radioimmunoprecipitation of purified p24 and gp41 derived from density gradient banded virus. In the second system, competitive immunoassays were developed using recombinant DNA expressed HIV p24 and gp41. In an attempt to evaluate utility of these immunoassays in diagnosis, sera from patients with AIDS, AIDS related complex and asymptomatic seropositive, as well as clinically normal individuals were tested for antibodies to HIV. High titered antibodies to gp41 could be detected in 100% of virus-exposed individuals, whereas, only 50-70% of AIDS patients exhibited antibodies to p24. When subjected to an enzyme-linked immunoassay which detects presence of HIV antigens (predominantly p24), the lack of antibodies to p24 in the sera correlated with the presence of HIV antigen. Based on these observations, the antibodies to gp41 are the most consistent marker for evaluation of HIV exposure, whereas, antibody titers to p24 may indicate various stages of the disease.

R23.51

SERODIAGNOSIS OF HIV-1-INFECTIONS BY AN OLIGOPEPTIDE-ELISA. K.-O.Habermehl, B.Zorr, H.Hampl, H.Zeichhardt, & P.Willingmann. Institute for Clinical and Experimental Virology, Free University of Berlin, Germany.

The ELISA has been proven as a highly sensitive and specific method in HIV-diagnosis. Nevertheless, unspecific reactions resulting in false positive results occur due to impurities of the antigen preparations. This problem can be solved in principle by using defined synthetic oligopeptides representing well conserved epitopes from different structural proteins of HIV-1. A number of synthetic oligopeptides (synthesized by Dr.Frenzel, Biochrom) have been screened in different ELISAs for reactivity against HIV-positive sera or negative controls (blood donors or patients from routine virus-diagnostic). Four oligopeptides containing 21 aminoacids each representing different epitopes from the proteins p18, p24, gp41 or gp120 with an optimal serologic specifity were selected and combined as a mixture in an ELISA. It could be demonstrated that with 505 blot-confirmed HIV-positive sera a reactivity of 100 % could be obtained. 2729 out of 2735 negative sera were in this ELISA non-reactive, indicating a specificity of 99,8 %. The good specificity and sensitivity was underlined by a significant difference between the extinctions of the non-reactives and the reactives in so far as 97 % of the positive probes showed an extinction of > 1.95 and 99,3 % of the negatives an extinction of < 0.15.

R23.52

HUMAN T-CELL LYMPHOTROPIC VIRUSES: EVALUATION OF VARIOUS IMMUNOLOGIC TECHNIQUES FOR DIAGNOSIS OF VIRUS INFECTION. S. G. Devare, G. J. Dawson, R. A. Gutierrez, R. G. Allen, J. M. Casey, D. Desjarlais, J. P. Allain, R. H. Decker, Abbott Laboratories, North Chicago, IL U.S.A.

HTLV-III/HIV, the causative agent of acquired immunodeficiency syndrome (AIDS), has been shown to be transmitted through blood transfusions. Recent data indicate that HTLV-I, the virus associated with adult T-cell leukemia (ATL) and tropical spastic paraparesis, also can be transmitted through blood transfusions. In an attempt to evaluate the incidence of HTLV infection, we tested sera from normal blood donors, drug abusers, and patients with ATL or AIDS for viral antibodies by a) solid phase immunoassays using detergent disrupted HTLV-I and HTLV-III, and b) radio-immunoprecipitation of major internal antigens (p24) of HTLV-I and HTLV-III. The presence of specific antibodies either to HTLV-I or HTLV-III were confirmed by Western blot analysis and/or immunoprecipitation of metabolically labeled viral proteins from infected cells. These methods readily differentiated antibodies to HTLV-I from HTLV-III. Our studies indicate that there is a high incidence of HTLV-I as well as HTLV-III infections among drug abusers, and that simultaneous infection of more than one agent is common.

R23.53

IgM CAPTURE ENZYME IMMUNOASSAY FOR RAPID DIAGNOSIS OF MEASLES J. Stewart, D. Humphrey, L. Chester. Division of Viral Diseases, Centers for Disease Control, Atlanta, USA.

A measles IgM (MIgM) capture enzyme immunoassay (EIA) was developed with biotin-labelled horse anti-measles antibody that avoids the problems of competition by IgG and false positive results due to rheumatoid factor. The parameters of the assay were adjusted to give the highest ratios between IgM positive and negative sera. IgM specificity of the assay was confirmed by the use of sucrose density-gradient serum fractions. Fifteen sera containing rheumatoid factor were IgM negative. Of 31 acute-phase sera from persons with measles, 25 were IgM positive. The other 6 negative sera were collected within 3 days of onset. All 31 of the follow-up sera were MIgM positive except for 2 collected within 11 days of onset. Thus measles infection can usually be confirmed with a single serum or by a small volume of blood collected on filter paper early in illness. The capture assay could also detect MIgM in sera that react nonspecifically with control antigen in our standard indirect EIA for MIgM with solid phase measles antigen.

R23.54

IN VIVO EXPRESSION OF RUBELLA ANTIGENS ON HUMAN LEUCOCYTES; DETECTION BY FLOW CYTOMETRY. S O'SHEA*, D MUTTON+, J M BEST*, Department of Virology* and Paediatric Research+, The United Medical and Dental Schools of Guy's and St Thomas' Hospitals, London, United Kingdom.

Sequential samples of leucocytes were obtained from 4 volunteers following naturally acquired rubella and 5 persons immunized with RA27/3 rubella vaccine. Leucocytes were stained for membrane antigens using an anti-rubella monoclonal antibody and anti-mouse FITC conjuate. Using flow cytometry, rubella antigens were detected on the leucocytes of all 4 volunteers with naturally acquired rubella between 1 and 13 days after onset of illness. Viral antigens were expressed more frequently on the monocyte (9-51%) than the lymphocyte (<1-4%) and granulocyte (<1-3%) populations. Among the vaccinees, rubella antigens were detected on the leucocytes of 4 of the 5 volunteers between 5 and 12 days after immunization. The expression of viral antigens was more transient and the proportion of cells exhibiting rubella specific fluorescence considerably lower following vaccination (1-12%) than natural infection (9-51%).

Our results show that flow cytometry provides a rapid and sensitive analytical technique for detecting viral antigens on leucocytes from infected persons. Leucocytes may play an important role in the pathogenesis of rubella infection.

R23.55

MEASLES VACCINATION IN SCORESBYSUND, GREENLAND.
I.R. Pedersen, C.H. Mordhorst, H. von Magnus, G. Glickmann. Department of Medical Microbiology, University of Copenhagen, and Statens Seruminstitut, Copenhagen, Denmark.

Measles vaccination was performed in 1968 in Scoresbysund, an isolated district of Greenland with 500 inhabitants. More than 90% of the population, which never had been exposed to natural measles, was vaccinated with Schwartz live further-attenuated vaccine. The immunity status of this isolated population has been followed for 16 years after the vaccination by IgM, IgA and IgG ELISA, by HI test and by immunoblotting. Initially, 94%-100% seroconverted and about 60% of the vaccinated persons seem still to be adequately protected. A temporary increase in measles antibodies occurred in 64% of the examined persons 2-4 years after vaccination. As this finding is important with relevance to vaccine protection, it will be shown whether this is due to a measles infection or to polyclonal activation with other viruses.

R23.56

ANTIBODY-CAPTURE ELISA THAT USES ENZYME-LABELED ANTIGEN FOR THE DETERMINATION OF IMMUNOGLOBULIN M, A AND G ANTIBODY TO VARICELLA-ZOSTER VIRUS. A. van Loon, F. Heessen and J. van der Logt. Dept. of Medical Microbiology, University of Nijmegen, The Netherlands.

An antibody-capture ELISA that uses peroxidase-labeled antigen was developed for detection of IgM, IgA or IgG antibody to varicella-zoster virus (VZV). None of the sera from patients with other herpes virus infections including herpes simplex virus infection or with rheumatoid factor reacted positively. All 18 patients with varicella produced VZV-IgM and VZV-IgG, 17 also VZV-IgA. In contrast, all of the 19 patients with herpes zoster were shown to be positive for VZV-IgA and -IgG whereas only 13 of these reacted positively for VZV-IgM. Similarly to patients with HSV encephalitis it was found that the presence of VZV-IgA in cerebrospinal fluid was a useful indicator of VZV meningo-encephalitis. By antibody-capture ELISA VZV-IgG was detected predominantly in sera from patients with acute or recent VZV infection. Most of the sera from healthy laboratory personnel remained negative for VZV-IgG by antibody capture ELISA, while being positive in an indirect type of ELISA. These results indicate that an antibody-capture ELISA is not suitable for determination of VZV immunostatus but may be an useful assay for determination of a recent VZV infection.

R23.57

SEROLOGICAL VARIABILITY AMONG BYDV ISOLATES FROM SOME COUNTRIES OF THE MIDDLE EAST AND NORTH AFRICA. K.M.Makkouk[1], I.Barker[2], O.Arzam[1], J.Skaf[3], and S.Forde[2]. (1) ICARDA, Aleppo, Syria. (2) Rothamsted Experimental Station, Harpenden, England. (3) School of Agriculture, Univ. Damascus, Syria.

PAV- and MAV-like isolates of BYDV were detected in cereal samples collected from Lebanon, Morocco, Syria and Tunisia. Some isolates reacted strongly with PAV-specific monoclonal antibodies (MAC91) and did not react with RPV or MAV specific monoclonals produced against British isolates (L. Torrance, MAFF, Harpenden, England) by using indirect ELISA. The same group of isolates reacted with Inotech "F" polyclonal antiserum in direct ELISA which is typical of a PAV reaction. Another group of isolates, however, did not react at all with the British PAV-specific monoclonal (MAC91) antiserum but reacted strongly with a PAV monoclonal (MC 32-39) produced in the U.S.A. against an American PAV isolate (S.Wyatt, WSU Washington). Some field isolates (from Tunisia and Morocco) reacted weakly with a MAV-specific monoclonal antiserum (MAC M2) produced against a British MAV isolate, but did not react with Inotech "F" antiserum which is unusual. Likewise another group isolates which reacted with Inotech "F" antiserum in direct ELISA did not react with PAV-, RPV-, or MAV-specific monoclonals which is also unusual. The availability at present of a number of BYDV monoclonals will help to identify more BYDV types than what has been done on the basis of vector specificity alone.

R23.58

DIRECT USE OF CARRIER-BOUND SYNTHETIC PEPTIDES AS ANTIGENS IN HIV AND EBV-SPECIFIC ELISA TESTS AND FOR THE ELICITATION OF SPECIFIC ANTIBODIES. Susanne Modrow,[1] Brigitte Höflacher,[2] Ronald Mertz,[3] Hans Wolf[1]. [1]Max von Pettenkofer Institute, University of Munich, Munich, FRG, [2]Gesellschaft für Strahlen- und Umweltforschung, Neuherberg, FRG, [3]Genzentrum, Martinsried, FRG.

Since solid phase peptide synthesis was introduced by Merrifield, this method has been established in many laboratories and synthetic peptides have become widespread in their use in natural and medical science. In particular, synthetic peptides were used as specific antigens in diagnostic test systems for the detection of antibodies in patient sera. Monospecific or monoclonal antisera against peptide sequences can be raised in animal systems and are used for the characterization and identification of the native polypeptides in cellular and molecular systems and have proven to be of great value in immunological test systems. Chemical methods for synthesis were considerably improved and adapted to automated systems which allow high yields of the respective products. The consecutive purification steps however are still very expensive and time consuming. For the use in ELISA or RIA tests and for antibody production peptides need to be coupled again to plastic or protein supports which may lead to the destruction of secondary structure and a loss of antigenicity. To avoid these problems we have developed a system where side-chain protection groups can be removed without simultaneous cleavage of the synthesized peptide from polystyrene supports. Peptides from EBV and HIV-specific proteins were synthesized and used in very sensitive ELISA tests to screen patients' sera for specific antibodies. In addition, the carrier-bound peptides were shown to be potent antigens for raising antibodies in immunized animals.

R23.59

EVALUATION OF LATEX AGGLUTINATION KITS FOR ROTAVIRUS DETECTION. P. Morgan-Capner and A.S. Bryden. Department of Virology, Preston Infirmary, Preston. PR1 6PS. U.K.

Four commercial latex agglutination tests, Rota Screen (Mercia Diagnostics), Slidex Rota-kit (bio Merieux), Rotalex (Orion Diagnostica) and "The Wellcome" (Wellcome Diagnostics) for rotavirus detection were evaluated using electron microscopy (EM), an 'in-house' ELISA and polyacrylamide gel electrophoresis (page) for comparison.

RotaScreen detected 91% of specimens deemed positive, Slidex 76%, Rotalex 87% and Wellcome 88%. All positives were detected by PAGE, 99% by ELISA and 88% by EM. Of rotavirus-negative specimens, 1% gave false positive reaction with RotaScreen, 1% with Slidex, 3% with Rotalex but 0% with Wellcome. The overall non-specificity rate i.e. test and control latexes both agglutinated, were 1% RotaScreen, 14% Slidex, 2% Rotalex and 5% Wellcome. It was found that non-specific and false positive reactions could be removed by fluorocarbon treatment of the faecal extract.

R23.60

Detection of respiratory viruses in nasopharyngeal secretion by EIA: results of a 3-year study. D.Germann, K.Schopfer Institue of clinical microbiology and immunology, 9000 St.Gallen, Switzerland

We have used a technique based on immunological detection of antigens of respiratory syncytial (RSV), adeno, parainfluenza 1, 2 and 3 as well as of influenza A and B viruses in nasopharyngeal secretions to evaluate diagnostically more than 5000 patients presenting with respiratory tract infections. The patients included babies, infants and children under 16. In about 40% of all sick babies below the age of 3 months a definite viral infection could be established, and in approximately 25% of infants and children aged up to 4 years. RSV was most frequently observed, accounting for more than 50% of all infections. The next most frequent pathogens were parainfluenza type 3 (16%), adenovirus (12%) and influenza A (8%).

In addition the results of these antigen detection assays have been compared with those of concmitantly performed virus isolation in tissue cultures. This comparative analysis most impressively revealed the time saved by using the antigen detecting system: in only 6% a specific diagnosis was established on the basis of virus isolation within 24 hours, whereas the delay was equal or more than 8 days in 36% of all patients enrolled.

R23.61

COMPARISON OF MRC-5 AND PRIMARY RABBIT KIDNEY (RK) CELLS FOR HERPES SIMPLEX VIRUS (HSV) ISOLATION USING A SHELL VIAL (SV) CENTRIFUGATION METHOD. E. M. Peterson, B. L. Hughes, S. L. Aarnaes and L. M. de la Maza. University of California Irvine Medical Center, Orange, California, USA.

A conventional tissue culture (CTC) method for the isolation of HSV was compared to the SV centrifugation method for HSV detection. MCR-5 and RK were used in both the CTC and SV systems. CTC were incubated for 7 days with daily examination for cytopathic effect which was confirmed as HSV by a fluorescent (FA) monoclonal antibody. SV were inoculated, centrifuged and incubated for 16 to 24 hours and stained with HSV monoclonals (Syva Corp.) or a polyclonal rabbit anti-HSV sera (Difco Labs.) with a horseradish peroxidase (HRP) - chromagen system. A total of 303 clinical specimens were examined of which 82 (27%) were HSV positive by CTC. The overall comparison between the cell lines, culture and staining method for the 82 positive specimens was:

	No. Pos. (%)		
Cell Line	CTC	SV-HRP	SV-FA
RK	81(99)	77(94)	65(79)
MRC-5	74(90)	72(88)	67(82)

Of the 82 CTC positive specimens 2 were missed by both RK and MRC-5 SV-HRP and 12 were missed by both cell lines SV-FA. In summary, the most sensitive method for detecting HSV was CTC with RK cells followed by RK cells in SV stained by HRP.

R23.62

ANTIBODY TO THE MEMBRANE OF HUMAN FIBROBLASTS CORRELATE WITH PRIMARY HCMV INFECTION. M.G.Revello, E.Percivalle, M.Zavattoni, G.Gerna.
Virus Lab, Inst. of Infectious Diseases, University of Pavia and IRCCS Pol. S.Matteo,27100 Pavia, Italy

Based on previous findings, 560 patients aged 3-40 with symptoms compatible with a primary human cytomegalovirus (HCMV) infection were tested for antibody to the membrane (MeAb) of uninfected humar embryonic lung fibroblasts by anti-complement immunofluorescence. In 45 MeAb-positive patients,an ongoing primary HCMV infection was diagnosed by seroconversion, presence of HCMV-specific IgM,and virus isolation. Correlation between MeAb and primary HCMV infection was not observed in patients younger than 3. MeAb (often associated to RF and ANA) possibly represent an aspect of the non specific humoral immune response. Determination of MeAb appears to be a new, useful parameter for screening of patients undergoing a primary HCMV infection.
Partially supported by the Min. San. IRCCS Pol. S. Matteo, Ric. Fin. 1985 "Tutela materno-infantile", and by the CNR grant No. 86.01625.52.

R23.63

PREVALENCE OF ANTIBODY TO ATYPICAL ROTAVIRUSES IN HUMANS.D.H. Dimitrov, L.M. Shindarov, S.M. Rangelova. Department of Virology, Institute od Infectious and Parasitic Diseases, Sofia, Bulgaria.

Until now in Bulgaria we found a total of 15 cases of viral gastroenteritis caused by rotaviruses which do not possess the common group A antigen and when analysed by PAGE they showed atypical migration profiles which appear to be identicalof human rotaviruses group C. In Bulgaria atypical rotaviruses were found in 1.8% of the samples tested by PAGE from 810 infants positive for rotaviruses.

To ascertain the role of atypical rotaviruses the presence of antibody to them was determined together with the presence of antibody to typical rotaviruses in infants. The antigens used were frozen sections of small intestine of piglets infected by direct inoculation of purified from fecal samples atypical and typical rotaviruses into ligated sections of the small intestine. The fixed antigens were detected by indirect immunofluorescence. Ten of the 42 test sera (21%) contained antibody to atypical rotaviruses and 35 sera (83%) contained antibody to typical rotaviruses. Experiments continue and it is likely that atypical rotaviruses play more significant role in the aetiology of human diarrhoea.

R23.64

FURTHER EVIDENCE OF THE SIGNIFICANCE OF SPECIFIC SERUM IgA ANTIBODIES AS A MARKER OF ACTIVE VIRAL AND CHLAMYDIAL INFECTIONS BY IMMUNOBLOTTING. I.Sarov, B.Sarov, N.Hanuka, A.Porath, B.Piura, E.Lunenfeld and P.Csango. Virol. & Epidem. Units & Div. of Internal Medicine & Gynec., Ben-Gurion Univ. of the Negev, Beer Sheva, Israel, and Central Hospital, Kristiansand, Norway.
Previous studies in this laboratory have demonstrated that specific serum IgA antibodies can be used as a marker of active primary and recurrent CMV and VZV, mumps and measles infections and in a number of acute and chronic chlamydial infections (reviewed by Sarov et al, New Horizons in Microbiol. 157, 1984). With highly sensitive immunoblotting techniques, we have recently detected, in 15 CMV mononucleosis patients, antibodies to 66-70K, 82K, 110K and 150K polypeptides; the most predominant being to 66-70K and 150KD. In 9 asymptomatic women and 10 men with nongonococcal urethritis (isolation positive), 50 women with salpingitis and 50 women with mechanical infertility, serum IgA antibodies specific for at least 18 chlamydial structural polypeptides (29K-204KD) were detected, the most predominant being antibody to 57K and 60-62KD). Serum IgA antibody to predominant antigenic components might be of particular importance in early recognition of persistant viral and chlamydial infections.
This study was supported by the Chief Scientist's Bureau, Ministry of Health, Israel, and the Middle East Eye Research Institute (MEERI).

R23.65

DETECTION OF ROTAVIRUS ANTIBODY IN NORMAL HUMAN SERA BY ELISA. Huilian Wu, Jinlin Guo, Yukun Zhang, Hong Meng. Wan Qing Han.
Institute of Basic Medical Sciences,Shandong Academy of Medical Sciences, Jinan, China.

277 serum samples collected from healthy persons of eight different age groups in Jinan area were tested by ELISA for the presence of Rotavirus (RV) antibody. The positive rate increased gradually (83.8-100%) with age from one year to adulthood. The lowest rate (51.5%) was in the 6 months to one year age group and 53.0% in the group of less than 6 months. Eighty-two of the 277 samples were determined both by ELISA and complement fixation test (CFT). In ELISA the positivity was 92.68% and its GMT was 894.6. The concordance rate of the two methods was 92.4%. The correlation coefficient (r) was +0.67. However, the titer of RV antibody in the sera determined by ELISA was 110 times higher than that by CFT. It means that ELISA is more sensitive than CFT. The result of blocking test further confirmed the specificity of ELISA method. Therefore, it is a simple, rapid and reliable method to be used in clinical diagnosis and epidemiologic investigation of RV infection.

R23.66

IMMUNOGOLD CYTOCHEMISTRY OF ALFALFA MOSAIC VIRUS IN ALFALFA GAMETES. Z. Pesic[1], M.H. Chen[2], C. Hiruki[1]. Department of Plant Science[1] and Department of Medical Microbiology[2], University of Alberta, Edmonton, Alberta T6G 2P5, Canada.

The indirect immunogold labelling technique was used to detect alfalfa mosaic virus (AMV) in ultrathin sections of ovules, pollen and anthers of three clones of alfalfa cv. Beaver infected with AMV (A-515). Anthers and ovules were collected from fully developed, untripped flowers from infected and virus-free plants grown in the greenhouse at 18 C and 16 h of photoperiod.

The TEM examination of sections prior to immunogold application revealed the presence of particle aggregates in anther parenchyma cells and pollen grains, but not in ovules. Using the colloidal gold IgG conjugate technique, viral antigen was detected in ovule integument cells, pollen and anthers. In all cases, AMV was found in the cytoplasm only. The specificity of antigen labelling with gold was confirmed with AMV in purified preparation. Sections of virus-free tissue treated by the standard procedure and sections of infected tissue exposed to normal rabbit serum showed no label under the conditions of this investigations. This is the first report of detection of AMV in alfalfa ovules.

R23.67

ELISA FOR EVALUATION OF ANTI-RABIES VACCINATION EFFICACY IN CATTLE. D. Katz, E. Freeman, M. Davidson*, M. Abramson*, O. Galan and P. Fuchs, Dept. of Virology, Israel Institute for Biological Research P.O.Box 19, Ness-Ziona and *Veterinary Services and Animal Health, Israel Ministry of Agriculture, Beit-Dagan, Israel.

The "Rabies Regulations" in Israel enforces a 6 months quarantine on any herd exposed to rabies, causing heavy economic losses to the farms involved. Determination of the immune status of the suspected animals might prevent these losses.

We have developed an ELISA for the detection of anti-rabies virus antibodies in cattle and studied the antibody response to the "Rabicine" inactivated vaccine (Merieux) in cows and calves following 1 and 2 dose vaccination schedules. The results obtained from a total of 261 sera indicate that: 1. The ELISA developed is suitable for use at regional veterinary stations; 2. The test has a good interassay reproducibility of ±20% ; 3. One dose of the vaccine induced a low antibody response in calves and a better response in cows. A booster vaccination significantly improved the antibody response both in calves and cows; 4. In all cases a rapid decline of anti-rabies antibodies (within 6 months) was observed.

R23.68

EARLY DIAGNOSIS OF HEMORRHAGIC FEVER WITH RENAL SYNDROME BY DEMONSTRATION OF SPECIFIC ANTIBODY OF IgM USING AN ELISA TECHNIQUES. Li-Li Chen, Hong-Xi Wang, Xian-Shi Gu, Shang-Zhi Chen, and Guang-Ming Qin.

Department of Viral Disease Control, Health and Antiepidemic Centre of Sichuan, Chengdu, People's Republic of China.

Demonstration of specific IgM antibody was carried out in sera from 58 patients with hemorrhagic fever with renal syndrome(HFRS) by an IgM ELISA with anti-μ coated solid phase. Measurements of 60 serial sera collected at different times after onset of the disease showed that the IgM antibody to HFRS virus(HFRSV) could be detected by day 5 and that the antibody titers revealed a peak by day 10 with a highest titer of 10^{-6}, rapidly declined after 30 and returned to negative after day 60. Qualitative assay of HFRSV IgM and total antibodies in 115 sera of the disease indicated that during the first five days of the disease the positive rate of HFRSV IgM antibody was 87.5 % and the rate of total antibodies determined by competitive ELISA was 58.0 %. The results of control tests showed that the HFRS IgM ELISA was without non-specific reactions and also eliminated the false positive reactions induced by the rheumatoid factor. Taken together, the technique has proven not only sensitive and specific, but also simple and rapid, thus being satisfactory for an early diagnosis of HFRS.

R24.1

PURIFICATION AND CHARACTERIZATION OF A VIRUS ASSOCIATED WITH PINEAPPLE MEALYBUG-WILT. D. A. Gunasinghe and T. L. German. Department of Plant Pathology, University of Hawaii, Honolulu, Hawaii, USA.

The presence of virus particles and dsRNA in association with mealybug-wilt of pineapple has been previously reported (Gunasinghe and German, 1986. Phytopathology 76:1073). Since that time the virus purification procedure has been improved by the addition of hide powder to the homogenization mixture, the use of reducing agents in the buffers, increased time of precipitation with ammonium sulfate and sucrose density gradient centrifugation. The increased purity of these preparations made further characterization possible. A particle length of approximately 850 nm has been determined. Antiserum has been prepared which reacts in Ouchterlony double diffusion assays only with sap from diseased plants or partially purified virus. The virus particles were "decorated" when the antiserum was used for immune electron microscopy.

R24.2

PLANT VIRUSES THAT RESEMBLE PICORNAVIRUSES. A.F. Murant, S.K. Hemida, M.A. Mayo. Scottish Crop Research Institute, Invergowrie, Dundee, UK, DD2 5DA.

Parsnip yellow fleck virus (PYFV) depends on anthriscus yellows virus (AYV) for transmission by aphids in the semi-persistent manner. PYFV is manually transmissible but AYV is not. PYFV occurs in many types of leaf cell whereas AYV is confined to phloem tissue. Dandelion yellow mosaic virus (DYMV) is also aphid-transmitted, probably in a semi-persistent manner, and is manually transmissible. All three viruses have isometric particles c. 30 nm in diameter. Each has a single polyadenylated genomic RNA of M. Wt ($\times 10^{-6}$) 3.3 to 3.5 (DYMV, PYFV) or 3.7 (AYV), and that of PYFV loses infectivity when treated with proteases, suggesting that it possesses a genome-linked protein. DYMV and PYFV each have three main particle proteins of M. Wt ($\times 10^{-3}$) c. 31, 29, and 26, or 31, 27, and 26 respectively. Immunoblotting and chemical cleavage experiments with both these viruses show that the three proteins of each differ in amino acid sequence. AYV too has particle proteins of M. Wt ($\times 10^{-3}$) c. 36, 30 and 22 . PYFV is proposed as the type member of a new virus group, with DYMV as an additional member. AYV may represent another virus group. All three viruses resemble the picornaviruses of vertebrates and insects in their particle properties.

R24.3

THE ASSOCIATION OF A NEW VIRUS-LIKE BARLEY DISEASE AND THE BROWN WHEAT MITE. N. Robertson, T. W. Carroll, Department of Plant Pathology, Montana State University, Bozeman, U.S.A.

A new disease of barley was discovered recently in Montana, U.S.A. Symptomatic leaves contained unique virus-like particles (VLPs) with widths between 66-74 nm and lengths varying from 150 to 4,000 nm; most of the VLPs appeared to be bounded by a membrane. No such VLPs were found in nonsymptomatic leaves. Tentatively the VLPs are considered to be the causal agent of the barley disease. However, no diseased plants resulted through mechanical inoculation and seed transmission tests and from leafhopper, aphid and thrips species collected from diseased fields and exposed to healthy plants.

In 1986, brown wheat mites, Petrobia latens Muller, were collected from diseased fields and placed on caged healthy barley seedlings. Also, mites were hatched in the laboratory from field collected diapausal eggs and fed on detached diseased or healthy leaves and then placed inside two separate cages with healthy barley seedlings, respectively. Plants in both experiments developed symptoms and the leaf tissues had the associated VLPs. Hence, these results suggest the possibility that the VLPs may be transovarially transmitted through the diapausal egg. The causal agent, therefore, may overwinter in the diapausal egg. Present studies on the VLPs, mites and plant host range are being conducted to fulfill Koch's postulates.

R24.4

PROPERTIES OF DNA AND PROTEIN ASSOCIATED SPECIFICALLY WITH FOLIAR DECAY DISEASE OF COCONUT PALM. R.G. Dietzgen, D. Hanold, P. Langridge, J.W. Randles, Departments of Plant Pathology and Agricultural Biochemistry, Waite Agricultural Research Institute, South Australia.

Foliar decay disease (FDD) of coconut palms in Vanuatu has an unknown etiology. A single-stranded circular DNA of mol. wt. ca. 0.4×10^6, comprising ca. 1300 nucleotides, can be isolated in small amounts from palms which show disease as a result of either experimental or natural infection. Two fragments of this DNA of about 200 and 350 nucleotides respectively have been cloned in the pUC19 plasmid of *E. coli*. ^{32}P-DNA probes prepared from this plasmid hybridize to a single component of the mobility expected for FDD-DNA in extracts from diseased palms only, confirming that the DNA is uniquely associated with infection, and indicating the potential diagnostic value of these probes. Antigen contained in fractions enriched with FDD-DNA was used to immunize rabbits and mice. Immunoblots of electrophoretically separated proteins from healthy and diseased palms using polyclonal antiserum and culture supernatants from hybridoma cell lines identified a single disease-specific antigen of mol. wt. 35kd.

A virus is therefore proposed as the cause of FDD, possibly comprising a 1.3 kb DNA and a 35 kd protein.

R24.5

VIRUS-LIKE ('CRYPTIC') PARTICLES PRESENT IN HEALTHY CEREAL TISSUE: PARTIAL CHARACTERIZATION AND PREPARATION OF SPECIFIC MONOCLONAL ANTIBODY. S. Haber, Agriculture Canada, Winnipeg, *and* A. Wieczorek, Agriculture Canada, Vancouver, Canada.

Virus-like particles were isolated in low yields (1-20 ug/kg) from leaf and root tissue of a number of lines of barley, oats and wheat grown under conditions that excluded the introduction of known viruses by seed, soil, water, contact or insects. Extracted particles separated by rate-zonal centrifugation into discrete peaks were spherical (13.5 nm/50 S; 18 nm/72 S; 25 nm/118 S), geminate (24 x 14 nm/72 S), or occasionally triplet in shape (36 x 14 nm/98 S). Geminate and triplet particles banded in gradients of cesium sulfate at 1.29 g*cm^{-3}; a $^{-3}$ band of 10%-shrunken particles formed at 1.31 g*cm. Removal of salt and reconcentration of equilibrium-banded fractions yielded only 18 nm spherical particles, showing geminate and triplet morphologies to be probable artefacts. Monoclonal antibodies were used to affinity-purify the 25 nm spherical particles which contained single-stranded (ss) RNA of $1.4*10^6$. Spherical 18 nm particles derived from fractions of geminate and triplet particles contained ssRNA of 1.4- and $0.72*10^6$.

R24.6

USE OF RESISTANT CUCUMIS METULIFERUS FOR SELECTION OF NITROUS-ACID INDUCED MILD MUTANTS OF PAPAYA RINGSPOT VIRUS. S.-D. Yeh and Y.-H. Cheng. Dept. Plant Pathology, National Chung Hsing University, Taichung, Taiwan

Papaya ringspot virus (PRV) HA 6-1, a nitrous-acid induced mutant that caused symptomless infection in papaya, was able to overcome the resistance in Cucumis metuliferus (Naud.) Mey. line 37, which is governed by a single dominant gene *Mmv*, and produced systemic mosaic and necrosis (SMN) symptoms. The possibility of using the resistant gene *Mmv* to screen useful mutants of PRV for control of the virus by cross protection was investigated. Virus in crude saps of susceptible C. metuliferus line 35 infected with PRV TH, a Taiwanese local strain, was treated with nitrous acid; enrich-cultured in line 35; and then transferred to the resistant line 37. Mutants that induced SMN symptoms on line 37 were selected and followed by repeated series dilutions on line 37. Pure lines of mutants were then transferred to papaya to investigate their pathogencity. Among 20 mutants selected, six induced mild symptoms on papaya; and one, designated as PRV TH-1, caused symptomless infection. Under greenhouse conditions, papaya inoculated with PRV TH-1 were completely protected against PRV TH when challenge inoculation was at 21 or 35 days after preimmunization. The results indicate that resistant C. metuliferus can be used as a selective host for screening useful attenuated mutants of PRV.

R24.7

MAIZE DWARF MOSAIC VIRUS INFECTING MAIZE AND SORGHUM IN EGYPT.

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The virus isolated from sorghum plants, showed severe mosaic and dwarfing, was identified as Maize Dwarf Mosaic Virus (MDMV) according to host range, stability, mode of transmission, particle morphology and serological reaction of the virus.
The virus was found to infect, mechanically, twenty one var.& cv. belong to family gramineae under glass house conditions.
The DEP, TIP and LIV were 1:500, 55°C and 24 hrs. respectively.
Experiments concerning mode of transmission indicated that, the virus isolate is transmitted mechanically and by aphid (R.maidis, R.padi and M.persicae)but was not transmit through mature maize and sorghum in their fullripe stage. The virus was found to be exist in seeds of maize and sorghum in milk and dough ripe stages.

R24.8

FINE, HELICAL, FILAMENTOUS STRUCTURES ASSOCIATED WITH MAIZE YELLOW STRIPE, A LEAFHOPPER-BORNE DISEASE AGENT FROM EGYPT.

E. D. Ammar. Dept. of Economic Entomology, Faculty of Agric., Cairo Univ., Giza, Egypt; Visiting Prof., Dept. of Plant Pathology, The Ohio State Univ., OARDC, Wooster, OH, USA.

Maize yellow stripe (MYStp), a disease infecting maize and wheat plants, transmitted persistently by the leafhopper *Cicadulina chinai* (Cicadellidae, Homoptera), has been recently reported from Egypt. Electron microscopy of thin sections from maize leaves, naturally or experimentally infected with the agent of MYStp, revealed large intracytoplasmic masses of fine filaments ca. 5-6 nm thick. In most cases, these filaments appeared to be helically wound to form long, flexuous tubular structures ca. 30 nm in diameter. Masses of these structures were detectable by light and electron microscopy in the phloem elements, vascular parenchyma and mesophyll of infected leaves, but were never found in healthy leaves.

The above described structures apparently have not been previously associated with any virus or viruslike disease in plants. The nearest comparable structures found in plant virus infections are the fine filaments associated with the Rice Stripe Virus group, some of which have spiral or supercoiled configurations. However, the cytopathology of MYStp in maize cells is markedly different from that reported for members of that group; additionally, these members have delphacid, rather than cicadellid, insect vectors.

R24.9

HISTOPATHOLOGY OF TOMATO PLANT INFECTED WITH A HEAT RESISTANT STRAIN OF TOBACCO MOSAIC VIRUS.

J.K.Makaroun, E.T.Sayed, M.M.El-Shamy. Department of Botany, Faculty of Science, University of Cairo, Giza, Egypt.

Tomato plants Lycopersicon esculentum cv. Money maker has been found to be naturally infected by a virus disease in a farm in Menoufia, Egypt. The virus induced mosaic mottling, blistering and distortion of the leaves. Identification studies have led to the conclusion that the virus may be a strain of TMV. Boiling the crude and purified virus for 10 min at 100 °C did not cause complete destruction of virus infectivity, in addition, it resisted at 100 °C for 31 min.

The histopathology of root, stem and leaves infected with the virus was studied in T.S. of different parts of the organ. The blade shows inconstant thickness. The cells of the spongy tissue are greatly undulated, and those of mesophyll may disintegrate leaving the upper and lower epidermis unaffected. A complete second leaf may develop on the upper or lower surface of the original leaf. Infection caused the presence of different shapes of epidermal cells and glandular hairs, of crystals in cells of epidermal hairs, cortex and vascular tissue of root, stem and petiole. The virus showed a marked effect on the vascular tissue of root, stem and leaf.

R24.10

PROPERTIES OF A VIRUS ASSOCIATED WITH AN ISOLATE OF BYDV.

M. Eweida, K. Tomenius, & P. Oxelfelt, Dept. of plant and forest protection, Swedish University of Agricultural Sciences, Uppsala, Sweden.

Preparations of an isolate of BYDV transmitted by Rhopalosiphum padi and serologically closely related to the American PAV isolate were found to contain spherical virus-like particles sedimenting at 150 S. In SDS-polyacrylamide gels the particles yielded four major protein bands ranging from 15000 to 32000 daltons. Aphid-transmitted viruses with similar sedimentation properties and protein composition have been described by Murant et al. (Scottish Crop Res. Inst. Ann. Rept 1985). A possible serological relationship between two of those viruses and the 150 S particles associated with BYDV is being investigated.

R24.11

PRIMARY STRUCTURE AND TRANSLATION OF THE GENOME OF SATELLITE TOBACCO MOSAIC VIRUS.

T. E. Mirkov, R. A. Valverde, D. H. du Plessis, and J. A. Dodds. Dept. Plant Pathology, University of California, Riverside, CA 92521, USA.

An icosohedral (T=1, diameter=17nm) satellite virus (STMV) was detected which depends for its replication on mixed infection with a tobamovirus (including TMV-U1 and TMV-U5). Ds cDNA synthesized by random priming of the ssRNA (MW=0,38 x 10^6) of STMV was digested with the restriction enzyme Taq I and inserted into the Acc I site of pUC19. Seven classes of STMV specific inserts were detected and subcloned into a sequencing vector (pUC119). Approximately 50% of the estimated 1000 bases of the genome has been sequenced from these fragments, and no homology to the sequence of TMV has been detected. This confirms the lack of homology determined by northern analyses between TMV RNA and STMV RNA using appropriate randomly primed ss cDNAs. A synthetic nucleotide sequence (5'-GGCCATCCCCGGGACGTC-3') complementary to the 3' terminal sequence of TMV was an efficient promoter of first strand cDNA synthesis from STMV RNA.

STMV ssRNA was translated in vitro and the major product was a polypeptide of the same size (18,000 daltons) as the STMV capsid protein. In western blot analyses this protein, capsid protein from purified virus, and capsid protein in sap from infected plants, was detected with antibody raised against STMV.

R24.12

CAPSID PROTEINS AND SEROLOGICAL COMPARISONS OF MAIZE RAYADO FINO (MRFV) AND RELATED VIRUSES.

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MRFV contains two capsid proteins, p22 and p28, present in unequal molar proportion of 9:1, respectively. Isolated p22 reaggregates to form multimers and spontaneously breaks down into smaller peptides of defined size. The p28 forms multimers to a very limited extent, is antigenically different from p22 and does not produce p22 polypeptides in gel reruns. Both polypeptides present similar peptide patterns in SDS-PAGE after limited proteolysis. We propose that in MRFV the closed shell is formed exclusively by p22 and that p28 is externally located, one per hexamer.

MRFV isolates from Costa Rica, Mexico, Colombia and Brazil and the African bermudagrass etched-line virus (BELV) were analysed by immunodifussion and immunoblotting tests. All isolates are serologically distinct strains. This virus and BELV are different but distinctly related viruses, both containing two capsid proteins. BELV occupies an intermediate position between MRFV and oat blue dwarf virus (OBDV) being closed to MRFV. OBDV and MRFV show a more distant relationship.

R24.13

MOLECULAR CHARACTERIZATION OF TOBACCO STUNT VIRUS.
S.A. Masri and C. Hiruki, Department of Plant Science, University of Alberta, Edmonton, Alberta T6G 2P5, Canada.

Tobacco stunt virus (TSV), a soil-borne virus that is transmitted by a chytrid fungus, Olpidium brassicae, causes severe stunting in certain cultivars of tobacco (Nicotiana tabacum) plants. The virus is rod-shaped measuring about 200-375 nm x 22 nm and contains RNA that behaves like a dsRNA. Analyses on 5% polyacrylamide gel electrophoresis revealed 3 molecular species of viral RNA; 4.2 x 10^6 daltons, 1.8 x 10^6 daltons, and 1.5 x 10^6 daltons. The RNA species of 4.2 x 10^6 daltons was detected in TSV-carrying zoospores by cDNA hybridization, but not in TSV-free ones. The RNA was resistant to degradation by RNase A1 in 2 x SSC and sensitive in 0.001 x SSC. It exhibited a sharp melting profile with a Tm of 62 C in 0.01 SSC and 75 C in 0.1 x SSC. Analyses of total TSV-RNA revealed a G:C ratio of 1.02 and a G + C content of 55.2%. In vitro translation of TSV-RNA in wheat germ extract produced 3 polypeptides; 67 K, 51 K and 28 K. The 51 K polypeptide was precipitated with TSV-antiserum and was identified as TSV coat-protein. These results suggest that TSV is different from tobamoviruses and 'furoviruses'.

R24.14

PEA PIMPLE POD DISEASE IS ASSOCIATED WITH AN APHID-BORNE VIRUS WITH PROPERTIES SUGGESTIVE OF A PLANT REOVIRUS.
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The agent of pea pimple pod disease is readily transmitted by the aphids Macrosiphum euphorbiae and Myzus persicae. These aphids transmit the pathogen from nasturtium (Tropaeolum majus) to pea (Pisum sativum), Datura stramonium and crimson clover (Trifolium incarnatum) in a persistent manner.

Double stranded RNA (dsRNA) analysis of infected material by PAGE resulted in two patterns. Pea, nasturtium and aphids showed nine common dsRNA bands of equal intensity, ranging in size from 2.6 to 0.7 Md, whereas Datura and crimson clover showed an array of bands of a different pattern. Thin sections of plant and aphid tissues revealed virus-like particles of 60 nm diameter, associated with viroplasms.

These results suggest that a plant reovirus is involved in the pea pimple pod disease, although the dsRNA pattern and the transmission by aphids are novel for plant reoviruses.

R25.1

ELECTRON MICROSCOPIC OBSERVATIONS ON THE PRESENCE OF RESPIRATORY SYNCYTIAL VIRUS ENVELOPED PARTICLES WITHIN CYTOPLASMIC INCLUSIONS IN INFECTED VERO CELLS.

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Electron microscopic examination of respiratory syncytial virus infected Vero cells revealed the presence of membranes delimited cytoplasmic inclusions and occasionally the synthesis of enveloped virus particles within these inclusions. These particles were enveloped, about 100 nm in diameter and of variable length, and contained nucleocapsid of about 12 nm. They look morphologically like the bacillary form particles which buds from the cytoplasmic membrane.

R25.2

CHARACTERIZATION OF A SYNTHETIC PEPTIDE THAT CARRIES A MAJOR NEUTRALIZATION EPITOPE OF HUMAN RESPIRATORY SYNCYTIAL VIRUS.

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During the last three years, our knowledge of the molecular biology of respiratory syncytial virus (RSV) has progressed tremendously. Several monoclonal antibodies have been isolated and used to characterize the different viral proteins. Moreover, the glycoproteins G and F of the A2 strain of RSV have been cloned, sequenced and expressed in vaccinia, identifying the major role played by the fusion glycoprotein in immune protection. By peptide mapping with V8 protease, we have identified, by immunoblotting with a neutralizing monoclonal antibody, a 7 kDa fragment of the fusion protein of the Long strain of RSV that retained a strong neutralization epitope. By amino acid analysis of this peptide we have assigned it to a specific region of the A2 published sequence. To confirm this localization, we have prepared several synthetic peptides to this region, 15 to 20 amino acids in length, one of which reacted with our neutralizing monoclonal antibody in neutralization inhibition and dot blotting assays. We are in the process of evaluating the immunogenicity of this synthetic neutralization epitope. Analysis of other members of the pneumovirus genus by neutralization and plaque reduction assays showed conservation of this epitope on bovine and caprine RSV and its absence on pneumonia virus of mice.

R25.3

SEQUENCE COMPARISONS OF RINDERPEST VIRUS STRUCTURAL PROTEIN GENES WITH THOSE OF OTHER MORBILLIVIRUSES.

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Rinderpest virus (RPV) or cattle plague is a serious, often fatal disease of cattle and other large ruminants. The disease is endemic in many parts of Africa, the Middle East and India. Great variation in pathogenicity is found with different isolates of the virus and the molecular basis for this strain variation is unknown. We have obtained cDNA clones of five of the six structural genes of the virus (N,P,M,F, and H genes). We are at present carrying out sequence analysis on these cDNA clone to determine the genetic relatedness of RPV to the other morbilliviruses. It is hoped that by sequence analysis of several strains with different pathogenecities that we may be able to understand the molecular basis of pathogenicity.

R25.4

SEQUENCE COMPARISON OF THE CLEAVAGE-ACTIVATION SITE OF FO PROTEIN OF VIRULENT AND AVIRULENT STRAINS OF NEWCASTLE DISEASE VIRUS (NDV).

T. Toyoda and Y. Nagai, Nagoya University School of Medicine, Nagoya 466, Japan.

Efficient proteolytic activation of the Fo protein in different cells, which is characteristic of virulent NDV strains, enables the virus to disseminate throughout its host and causes extensive diseases, whereas the Fo protein of avirulent strains, which show a narrow tissue specificity, is not cleaved in many cells. A single open reading frame of the Fo mRNA of a virulent strain encodes a protein (553 amino acid residues) with several structural features involving the fusion-inducing hydrophobic sequence (PIGAIIGSVALGVATAAQI-TAASALI) and the cleavage-activation site (RRQRR). The same dibasic sequences with an intervening glutamine was found at the cleavage site of all the virulent strains analyzed, whereas the single basic residues together with uncharged residues ($G^{R}_{K}Q^{G}_{S}R$).

Thus the importance of dibasic sequence was suggested for the efficient proteolytic activation of Fo protein and pantropic property of NDV.

R25.5

RESTRICTION OF MEASLES VIRUS N AND F PROTEIN SYNTHESIS AT ELEVATED TEMPERATURES.

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Recently, we reported that the synthesis of N protein of measles virus is restricted at 39°C at which temperature the other viral proteins are still synthesised. There is a similar restriction in the synthesis of F protein at 39°C. The effect appears not to be related to the synthesis of the mRNA or its stability after temperature shift nor is it related to the stability of the protein at 39°C. It is clear that temperature shift blocks the translation of the mRNAs encoding the N and F proteins and that this inhibition is immediately reversed when the temperature is shifted back to 35°C. Experiments with initiation and elongation inhibitors and pulse-chase experiments indicate that the elongation of the N and F proteins is affected by the temperature shift. Polysomes populations were analysed from cells incubated at 35°C or at 39°C. There was very little difference in the distribution of the N and F mRNAs over a gradient on which the polysomes were size-fractionated. Cell fractionation and use of drugs that affect the cytoskeleton indicate that this is not involved in the observed phenomenon. Experiments with cold-sensitive mutants indicate that their N protein synthesis is somewhat less sensitive to temperature elevation than that of other measles virus strains.

R25.6

A NOVEL METHOD FOR MAPPING VIRUS EPITOPES

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Monoclonal antibodies (Mabs) which react with the haemagglutinin-neuraminidase (HN) glycoprotein of Newcastle disease virus (NDV) were grouped according to various criteria (i) the sensitivity/resistance of mutants selected to be resistant to individual neutralizing Mabs against the remaining Mabs, (ii) the reactivity of HN proteins from Mab-resistant mutants to the set of Mabs as determined by "Western blot" analysis and (iii) the reactivity of wild-type HN partial digest fragments (produced by a variety of proteases and protein cleavage reagents) to Mabs detected by "Western blot" analysis.

The latter technique, developed in this laboratory, has the advantage over others that (i) only small quantities of Mabs are required, (ii) antibody-resistant mutants need not be isolated, and (iii) given the protein sequence (NDV HN sequence see Miller et al J. gen. virol. 67; 1917-1927 (1986)), it is possible to make predictions as to which region(s) of the protein a given Mab binds.

R25.7

PARAMYIOVIRUS METABOLISM ASSOCIATED WITH THE CYTOSKELETAL FRAMEWORK.
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Research Institute for Disease Mechanism and Control, Nagoya University School of Medicine, Nagoya 466, Japan.

We previously reported that the nucleocapsid protein(NP) of Newcastle Disease Virus(NCD) associate with the cytoskeletal framework(CSK) soon after synthesis and assemble there into nucleocapsid together with the viral genome RNA(VIROLOGY 147,295,1985). Treatment of the CSK of infected chick embryo cells with either 0.3MKI,3.5MTris-HCL pH8.0, or CSK buffer (0.1MKCL,2.5 mMMgCl$_2$,0.3MSucrose,10mMPIPES pH6.8) containing 1mMATP was found to release NP together with the cytoskeletal protein components. When the materials are further dialyzed against 0.1MKCL,1mMMgCl$_2$, and 0.1mMATP, the NP was found to form a filamentous complex together with the cellular actin, suggesting a specific interaction between the NP and actin. We also show the association of viral mRNAs with CSK.

R25.8

CLONING AND SEQUENCING OF A cDNA CORRESPONDING TO THE P PLUS M DICISTRONIC mRNA OF AN SSPE VIRUS. Y. Yoshikawa, H. Tsuruoka, K. Yamanouchi, T. Shioda, H. Shibuta. Institute of Medical Science, University of Tokyo, Tokyo, Japan.

The nucleotide sequence of a cloned cDNA corresponding to the P + M dicistronic mRNA of an SSPE virus was determined and compared with that of measles virus (MV). The part encoding the P gene had a high homology to MV except at the noncoding region. The R2 sequence, which is the terminating consensus sequence of each gene, and the intercistronic trinucleotides were CTAC(A)$_6$ and CCT; in MV they are TTAT(A)$_6$ and CTT, respectively. In the part encoding the M gene, the R1 sequence, which is the starting consensus sequence of each gene, had exactly the same sequence as MV, but the 5' proximal of this gene was different: the first ATG codon of the MV M gene was changed to ACG in the SSPE virus and one long open reading frame started from the third ATG codon. The stop codon (TAG) of the MV M gene was also changed to CAG in the SSPE virus. Thus, the deduced SSPE virus M protein lacked 50 amino acids at the amino terminal and had extra 15 amino acids at the carboxyl end when compared with MV M protein. The lack of several epitopes of SSPE M protein was confirmed by using monoclonal antibodies against MV M protein and reduced MW. corresponding to the deduced amino acid sequence was shown by a Western blotting method.

R25.9

RESPONSE OF INFANTS WITH BRONCHIOLITIS TO THE PROTEINS OF RESPIRATORY SYNCYTIAL (RS) VIRUS. S. Levine[1], A. Dajani[2] R. Klaiber-Franco[1]. [1]Department of Immunology & Microbiology [2]Department of Pediatrics, Wayne State University, Detroit, MI, 48201, U.S.A.

RS virus was isolated from 25 of 34 infants admitted to Children's Hospital of Michigan with the diagnosis of bronchiolitis. Acute phase sera, obtained within 48 hours of admission, and convalescent phase sera, obtained 2 to 4 weeks later, were assayed for neutralizing antibodies by plaque inhibition and for antibodies to the viral proteins G,F,N,P & M, by western blot against partially purified RS virus.

Of the 34 acute phase sera, 19 had neutralizing antibody to RS virus, 33 had antibody to glycoprotein G, the attachment protein, 21 to glycoprotein F, the fusion protein, 22 to N, the major nucleocapsid protein, 14 to P, the phosphorylated nucleocapsid protein, but none to M, the non-glycosylated envelope protein.

Only 4 of the 29 infants from whom a convalescent serum was obtained responded with an increased neutralization titer, while 10 responded to G, one to F and none to M. Surprisingly, at least 21 responded to P, a minor structural protein, suggesting that these infants, 18 of whom are younger than 6 months, had responded to the virus infection, but that the response to the other viral proteins was masked or aborted by residual maternal antibody to these proteins.

R25.10

SEQUENCE ANALYSIS OF HUMAN PARAINFLUENZA VIRUS 3 GENES ENCODING ENVELOPE ASSOCIATED PROTEINS. M.-J. Cote, D. G. Storey, K. Prinoski, T. Karnauchov, C. Y. Kang, K. Dimock. Department of Microbiology and Immunology, Faculty of Health Sciences, University of Ottawa, Ottawa, Canada.

Human parainfluenza virus 3 has three proteins associated with its envelope: the HN glycoprotein, which has both haemagglutinin (receptor binding) and neuraminidase activity; the F glycoprotein, which exists as a complex of two polypeptides, F_1 and F_2 and is responsible for virus penetration and cell fusion; the M or matrix protein which is believed to link the envelope with the nucleocapsid.

We have chosen a recombinant DNA approach to study the interactions between these three proteins and their respective roles in virus assembly and immunogenicity. As a first step, the complete nucleotide sequences of the coding and flanking regions of the HN, F and M genes of HPIV3 have been determined. Primer extension was used to identify the 5' termini of the HN, F and M mRNAs. Complete copies of each gene have been constructed and studies of their expression in monkey kidney cells are underway.

R25.11

CONSTRUCTION OF A cDNA CLONE REPRESENTING THE ENTIRE L GENE OF SENDAI VIRUS. E. M. Morgan, D. W. Kingsbury. Department of Virology and Molecular Biology, St. Jude Children's Research Hospital, Memphis, Tennessee 38101-0318, U.S.A.

The L gene, the largest of the Sendai virus genes, encodes a large protein (M_r = 231,608) that is centrally involved in viral RNA synthesis. Recently, we reported the complete sequence of the L gene obtained, in part, from a library of cDNA clones. Several of these clones, ranging in size from 1000 to greater than 3000 basepairs, form a nested set that encompasses the entire gene. We are assembling these segments into a complete cDNA clone to study structure-function relationships of the L protein.

R25.12

ANALYSIS OF PERSISTENT MEASLES VIRUS INFECTION IN RAT BRAIN CELLS.
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Inoculation of a neurotropic measles virus into Lewis rats leads to a subacute encephalomyelitis (SAME) after an incubation period of 2-3 weeks on the basis of a persistent infection. Analysis of this persistent infection in brain tissue reveals already at onset of the disease a restriction of measles virus gene expression, affecting the envelope genes. By immunohistochemical studies using monoclonal antibodies to the different measles virus structural proteins, N and P were always detectable in infected brain cells while H, F or M protein were either reduced or absent, when compared with lytically infected cells. mRNAs for all these proteins were found in brain cells, however, those for the envelope proteins were reduced. In in vitro translation experiments, an impaired synthesis of envelope proteins were observed indicating a defect in translation. These findings are similar to the ones recently observed in subacute sclerosing panencephalitis (SSPE) in which mutational changes have been found to be responsible for the restriction of measles virus gene expression. However, in our animal model these changes occur already a few weeks after infection suggesting that besides possible accumulation of mutations host factors may play a pathogenetic role.

R25.13

ISOLATION AND STRUCTURAL ANALYSIS OF NEWCASTLE DISEASE VIRUS PROTEINS.

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Disrupted Newcastle disease virions (Queensland and Australia -Victoria) have been subjected to a procedure which labels their protein constituents with a fluorophor. (J.J. Gorman; Anal. Biochem. In Press). Attachment of this fluorophor enables detection of very low levels of proteins on SDS-acrylamide gels. In addition, because the fluorophor is attached via cysteinyl residues the proteins are amenable to extensive primary structural analysis.

Fusion protein components (F1 and F2), the haemagglutinin/neuraminidase, nucleocapsid and matrix proteins of Newcastle disease virus have been isolated by electroelution from a single SDS-acrylamide gel and subjected to gas-phase sequence analysis, peptide mapping and mass spectrometric analysis. Of the proteins investigated only the fusion protein components had free amino termini. The amino terminus of the F2 generated by signal peptidase cleavage was found to begin with Leu-Asp-Gly-Arg-. The amino terminal residue of the F1 component of the fusion protein of the Queensland Strain differs from that of the Australia-Victoria strain and other paramyxoviruses. The implications of this variation to the pathogenicity of Newcastle disease viruses will be discussed.

R25.14

DEFECTIVE INTERFERING PARTICLES OF HUMAN PARAINFLUENZA VIRUS 3. D. G. Murphy, K. Dimock, C. Y. Kang. Department of Microbiology and Immunology, University of Ottawa School of Medicine, Ottawa, Canada.

A cyclic pattern of virus production was observed when human parainfluenza virus 3 (HPIV3) was serially passaged nine times in LLC-MK2 cells. Virus produced from serial passages 8 and 9 interfered with the replication of standard HPIV3. Three subgenomic RNA species (DI-1, DI-2 and DI-3) and the standard virus genomic RNA were detected in progeny virions produced from cells mixedly infected with passage 8 virus and the standard virus. Northern blot analysis with probes representing all six HPIV3 structural protein genes revealed that DI-1 and DI-2 RNAs contained sequences from the 5' end of the standard virus genome. DI-1 RNA contained L, HN and F specific sequences while DI-2 RNA contained L and HN sequences only. DI-3 RNA may well represent the 5' end of the L gene sequence. These results demonstrate that 5' defective interfering (DI) particles are generated during serial passage of HPIV3.

Infection with a mixture of standard virus and DI particles of LLC-MK2 cells readily leads to the establishment of a persistent infection. Standard size genomic RNA and subgenomic RNAs were present in virions released from the persistently infected cells. The characterization of these subgenomic RNAs will be presented.

R25.15

NUCLEOTIDE SEQUENCE ANALYSIS OF THE HUMAN PARAINFLUENZA 3 VIRUS. M.S. Galinski, M.A. Mink, D.M. Lambert, S.L. Wechsler, and M.W. Pons.

The molecular organization of the human parainfluenza 3 virus (PF3) has been determined by nucleotide sequence analysis of recombinant plasmids. The molecular cloning and sequence analysis has provided the complete gene sequences and coding strategy for all but one of the major structural proteins/genes including the nucleocapsid protein, NP, the nucleocapsid-associated phosphoprotein, P, and C proteins, the membrance associated fusion protein F, and the hemagglutinin-neuraminidase protein, HN. In addition, the intergenic regions between all the encoded genes have been determined. These results have allowed us to order the genes as 3' -N-P/C-M-F-HN-L-5'.

R25.16

MEASLES, MUMPS AND RESPIRATORY SYNCYTIAL VIRUS SHOW T CELL CROSS-REACTIVITY. BARRY ZIOLA, RH SMITH. Immunovirology Research Group, Dept. of Microbiology, Univ. of Saskatchewan, Saskatoon, Sask., Canada S7N 0W0

Measles, mumps and respiratory syncytial (RS) virus have little or no antibody cross-reactivity, which fits with these viruses being put into different genera within the paramyxoviridae. The question of whether these 3 viruses (genera) remain distinct at the level of T cell responses has not been answered. This is despite T cell responses being vital in clearing enveloped virus infections.

We have used mice pretreated with cyclophosphamide and immunized with inactivated virus antigen in dimethyl dioctadecyl ammonium bromide to study T cell responses to measles, mumps and RS virus. In both delayed-type hypersensitivity assays and in vitro blastogenesis assays, these 3 viruses have shown extensive T cell cross-reactivity. No T cell relationship to herpes simplex 1, vesicular stomatitis or influenza PR8 virus was found, demonstrating the specificity of the results.

It now becomes important to determine whether a previous infection with RS virus (or perhaps a parainfluenza virus) could influence subsequent development of immunity to measles and mumps virus commonly given as attenuated vaccine viruses.

Supported by grants from the Medical Research Council of Canada.

R25.17

SYNTHESIS AND PROCESSING AND STRUCTURAL ANALYSIS OF RESPIRATORY SYNCYTIAL VIRUS GLYCOPROTEINS. Dennis M. Lambert. Department of Molecular Virology, James N. Gamble Institute of Medical Research, Cincinnati, OH, USA 45219.

Human respiratory syncytial (RS) virus grown in CV-1 cells, contains two surface glycoproteins, a 84,000 dalton protein (G) and a 70,000 dalton protein (F). The fusion protein, F, consists of two disulfide-bonded subunits of approximately 48,000 (F_1) and 21,000 (F_2) daltons. Glycosylation of the G protein is partially resistant to tunicamycin (TM), an inhibitor of N-linked glycosylation, suggesting that the G protein contains both N-linked and O- linked carbohydrates. Pulse-chase experiments showed that the F protein was synthesized as a 68,000 dalton precursor glycoprotein which was cleaved to its subunits within 15-30 minutes after synthesis. Effects of a number of glycosylation inhibitors on processing of these glycoproteins were examined. Processing of the N-linked oligosaccharides of G protein was affected by TM, monensin, swainsonine and bromoconduritol. The effects of these drugs on RS virus infectivity were examined and results suggest that proper glycosylation and processing of the oligosaccharides of G and F proteins are necessary for infectivity. The normal oligosaccharides of purified F and G proteins were removed both chemically and enzymatically and analyzed by BioGel P6 chromatography.

R25.18

IMMUNOLOGIC REACTIVITY OF MEASLES VIRUS POLYPEPTIDES. J. Rose, S. Jacobson, J. Richert, J. Scales, E. Minigioli, M. Fleriage, H. McFarland and D. McFarlin. Neuroimmunology Branch NIH and the University of Utah School of Medicine. Preparation of measles virus (MV) polypeptides by affinity chromatography with monoclonal antibodies resulted in antigenically pure proteins. Analysis of each isolated polypeptide in the ELISA with a panel of monoclonal antibodies demonstrated significant reactivity only with monoclonal antibody specific for that polypeptides and not anti-measles monoclonals with other specificities or a irrelevant monoclonal antibody. The isolated polypeptides retained antigenicity for both humoral and cell mediated immune responses. The polypeptides remained immunogenic as demonstrated by the generation of polyclonal IgG antisera in mice. The purity of the HA, F, and N preparations was further demonstrated by the fact that polyclonal antisera raised against the polypeptides precipitate only one viral protein. These antisera precipitated individual polypeptides from cell lysates, reacted specifically in the ELISA and specifically stained MV but not uninfected cells. Epitopes required for lymphoproliferation and generation of cytotoxic T-lymphocytes (CTL) were preserved. The affinity purified internal poly-peptides (NC and M) and the external polypeptides (HA and F) are capable of generating CTL specific for MV infected autologous cells.

R25.19

CLONING OF THE NUCLEOCAPSID (N) PROTEIN AND POLYMERASE-ASSOCIATED (P) PROTEIN GENES OF PESTE DES PETITS RUMINANTS VIRUS.

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Peste de petits ruminants virus (PPRV) is the fourth member of the morbillivirus group which includes measles and canine distemper virus. Small ruminants, including sheep and goats, are the natural hosts for the virus. Another virus in the group, rinderpest virus (RPV), mainly infects large ruminants. These viruses cause similar severe respiratory diseases in their natural hosts. The four viruses in the group are closely related antigenically but they can be distinguished biochemically and by hybridisation with cDNA clones specific for each virus. Both measles and canine distemper viruses can persist in their hosts and cause chronic neurological disease. It is not known whether RPV and PPRV can persist in infected animals and what, if any, significance this has for the epidemiology of the virus. We have obtained cDNA clones of two of the structural genes of PPRV, the N protein and the P protein genes, and we hope to use these to examine the genetic relationship of PPRV to the other viruses in the group and to examine the possibility that virus persists in the infected host after recovery from the acute disease.

R25.20

STRUCTURE AND POSSIBLE FUNCTIONS OF POLYADENYLATED RNA CONTAINING A LEADER SEQUENCE FROM MEASLES VIRUS-INFECTED CELLS
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The negative-stranded RNA genome of measles virus serves as a template for both genomic length antigenomes used in replication and mRNAs. Both forms of RNA synthesis initiate at the 3' end of the genome. Although the entire 3' sequence is represented in the antigenome, nucleotides 1-56 (the leader) are believed to be absent from mRNAs.

From an expressible cDNA library, we have isolated a clone indicating some polyadenylated RNA derived from the first gene (encoding nucleocapsid protein, N) contained a complete leader sequence. Sequence analysis of this clone revealed that transcription began at the 3' terminal end and accurately copied the viral genome through both the N and P (encoding phosphoprotein) genes before polyadenylation occurred. A quantitative S1 analysis comparing the ratio of N-encoding mRNAs with and without the leader indicated the former RNA species was present in significant amounts in infected cells. The possible function of this RNA species was studied by comparing in vivo expression of this clone to monocistronic cDNAs which encoded N or P proteins. These data suggested this RNA species might either reflect a transition from transcription to replication or indicate transcription can initiate at nucleotide 1.

R25.21

STRUCTURE OF THE RESPIRATORY SYNCYTIAL VIRUS GLYCOPROTEIN G
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The G protein of respiratory syncytial (RS) virus is responsible for attachment of the virus to the host cell. We have purified the G protein and analyzed its structure with particular emphasis on size and glycosylation. The predominant form of the protein has an apparent molecular weight of 90K as has been reported previously. A protein of 45K daltons consistently co-purifies with the 90K protein and is immunologically crossreactive based on analysis with monoclonal antibodies. Carbohydrate analysis of the G protein indicates a total carbohydrate content of 30-35% with galactose and N-acetyl galactosamine being the most comon sugars. Both the 90K and 45K protein contain both N- and O-linked sugars, based on reactivity with specific agglutinins and susceptibility to both O- and N-specific glycanases. The data suggest the possibility that the 45K protein is a monomeric form of the G protein comprised of 33K daltons of protein (based on the DNA sequence) and 12K daltons of carbohydrate.

R25.22

CHARACTERIZATION OF IN VITRO TRANSCRIPTION AND TRANSCRIPTIONAL PRODUCTS OF MEASLES VIRUS. R.S. Fujinami and J. Ray. Department of Pathology, University of California, San Diego, La Jolla, California, USA.

Ribonucleoprotein complexes isolated from measles virus infected HeLa cells contained an RNA-dependent RNA polymerase activity that catalyzed the incorporation of ribonucleotides into ribonucleic acid. The ribonucleoprotein complexes are composed of measles virus nucleoprotein, phosphoprotein and a large protein as well as viral RNA. The kinetics of RNA synthesis at different temperatures, time intervals, and protein, ribonucleotide, mono and divalent cation concentrations were analyzed. Enzyme activity was maximal at 4 h at 25°C in the presence of 100 mM Na^+, 2.5 mM Mg^{++} and 1 mM ribonucleotides. Actinomycin-D and α-amanitin had no effect on the enzyme activity. Addition of cytoplasmic extracts from uninfected HeLa cells to the reaction mixture did not increase the incorporation of ribonucleotide into RNA. The in vitro synthesized RNAs were characterized by slot blot analysis and quantitated by densitometer scanning. All mRNAs coding for the structural proteins of measles virus were synthesized. The nucleoprotein RNA was the most abundant species made, followed by phosphoprotein, hemagglutinin, fusion protein, matrix protein and large protein RNAs. The system described here resulted in the first efficient transcription of measles virus RNA and analysis of products. This work supported by NIH NS-23162 and GR NMSS 1780-A.

R25.23

Nucleotide Sequence Analysis of Hemagglutinin-Neuraminidase(HN) Gene and its precursor(HNo) Gene of Newcastle Disease Virus(NDV)
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The nucleotide sequences of the HN and HNo Genes of NDV were determined. The HN gene is 2031 nucleotides long, and contains a single long open reading frame which encodes a protein of 571 amino acids with a mol. wt. of 68217. There are 6 potential glycosylation sites. The major hydrophobic region was close to the N-terminus. The HNo gene has the same size as that of HN. However, its open reading frame which starts at the same initiation codon as that of HN, encoding a protein of 616 amino acids with a mol. wt. of 75671. This extended region of 45 amino acids of HNo contains a single glycosylation site and could be removed by proteolytic cleavage to convert HNo to HN. These data provides the basis of the strain-dependent genesis of either HN or HNo in NDV.

R25.24

ANTIGEN RELATEDNESS OF THE ENVELOPE GLYCOPROTEIN (GP) OF TWO RESPIRATORY SYNCYTIAL VIRUS (RSV) STRAINS. E.E. Walsh, M.W. Brandriss, J.J. Schlesinger. Department of Medicine, Rochester General Hospital, University of Rochester, Rochester, New York, USA.

Two prototype strains of RSV (18537 and Long) have recently been proposed, based upon reactivity with monoclonal antibodies (MABs). To further characterize these differences, the fusion (F) protein and large GP (G) of both strains were purified by affinity chromatography using MABS. Rabbit antisera to each protein was used to characterize the proteins by enzyme immunoassay (EIA), Western blot (WB), neutralization (N) and fusion inhibition (FI) assays. Purified F proteins had identical molecular weights (Mr) by SDS-PAGE, and were closely related immunobiologically by EIA, WB, N, and FI using polyclonal monospecific antisera (calculated relatedness* = 77-100%). Epitope mapping using a previously described panel of 13 MABs revealed that all three fusion inhibiting neutralizing epitopes were present on both F proteins. Conversely, 18537 G protein had a Mr of 78K, compared to 84K for Long G. Antiserum to 18537 G had comparative 18537 G/Long G titers of 25600/50 by EIA and 1280/20 by N and only reacted with 18537 G by WB, while antisera to Long G had comparative 18537 G/Long G titers of 400/51200 by EIA and 20/2560 by N and reacted preferentially with Long G by WB. Calculated relatedness between G proteins was 1-3%.
* relatedness calculated by Archetti-Horsfall formula

R25.25

SEQUENCES OF THE PROPOSED CLEAVAGE SITE OF THE FUSION GLYCOPROTEIN OF VIRULENT AND AVIRULENT STRAINS OF NEWCASTLE DISEASE VIRUS. R.L. Glickman, R.J. Syddall, R.M. Iorio, J.P. Sheehan, and M.A. Bratt. Department of Molecular Genetics and Microbiology, University of Massachusetts Medical School, Worcester, MA, USA.

Newcastle disease virus exists as both virulent and avirulent strains. Pathogenicity of the virus has been correlated with the susceptibility of the fusion (F) glycoprotein to cleavage by a cellular protease. A primer was synthesized from the deduced sequence of the F glycoprotein of strain AV (McGinnes and Morrison, Virus Research 5, 343), and using primer extension and "dideoxy" sequencing the nucleotide sequence around the proposed cleavage site of the HN glycoprotein was determined and compared in between several virulent and avirulent strains. The sequence in this region in virulent strains consists of four basic amino acids with an intervening glutamine (Arg-Arg-Glu$^{Arg}_{Lys}$-Arg). In contrast, the sequence of the F glycoprotein of avirulent strains differs in that one of the basic residues on either side of the glutamine is replaced by a neutral amino acid, usually glycine (Gly-Arg-Glu-Gly-Arg). This suggests a requirement for several basic amino acids at the cleavage site for proteolytic activation of the F glycoprotein of NDV in most cell types.

R25.26

PROTECTION AGAINST A LETHAL NEUROTROPIC STRAIN OF MEASLES VIRUS BY F AND H ISCOM COMPLEXES AND BY HEMAGGLUTININ-SPECIFIC MONOCLONAL ANTIBODIES. T.M. Varsanyi, *B. Morein, A.Löve and E. Norrby. Dept of Virology, Karolinska Institutet, Stockholm, Sweden and *Dept. of Virology, Faculty of Vet.Med. Nat. Vet. Inst., Uppsala, Sweden.

The potential of the individual surface glycoproteins of measles virus in active and passive protection was examined in a BALB/c mouse model. Infected cell lysates were depleted of the hemagglutinin (H) or the fusion (F) glycoprotein using immunoaffinity chromatography in a sequential way. The products were used to prepare the F or H protein containing immune stimulating complexes (iscoms). Groups of BALB/c mice were inoculated with the iscom preparations. Immunsera from the groups immunoprecipitated only the homologous glycoproteins. The animals were challenged with a lethal dose of hamster neurotropic (HNT) variant of measles virus, adapted to BALB/c mice. Fifty procent of the animals in the non-immunized control group died within 10 days after challenge, while the F iscom immunized group showed no symptoms of disease in the post-challenge period of two and a half months.This was also true for the H iscom immunized group but it showed some signs of overgoing disease in the first 10 days of the post-challenge period. Passive administration of hemagglutinin-specific monoclonal antibodies also gave full protection against the HNT virus in newborn mice.

R25.27

CHARACTERIZATION OF RESPIRATORY SYNCYTIAL VIRUS STRAINS: LIMITATIONS AND OPPORTUNITIES. LJ Anderson, JC Hierholzer. Centers for Disease Control, Atlanta, GA, USA.

To better understand antigenic variation among respiratory-syncytial virus (RSV) isolates, we reacted 25 monoclonal antibodies (MAb) against 60 group 1 and 9 group 2 isolates. These 25 MAbs represent 7 distinct epitopes on the F protein, 12 on G, 4 on N, and 2 on 22K Group 1 and 2 isolates could be distinguished by 1 F MAb that reacted with only group 1 isolates and 1 F MAb that reacted with only group 2 isolates. The greatest differences between the 2 groups were seen on G with 8 of the 12 MAbs only reacting with group 1 isolates. Only 2 of these 8 MAbs reacted with most (>95%) of the group 1 isolates; the other 6 reacted with 7% to 45%. One of the 4 G MAbs that reacted with both group 1 and 2 isolates reacted with most (>95%), while the other 3 reacted with 28% to 70% of the 69 isolates. Five of 7 F MAbs reacted with group 1 and 2 isolates (80% to 100%); the two 22K MAbs reacted only with group 1 isolates (38% to 47%); one N MAb reacted only with group 1 isolates (62%) and 3 reacted with both group 1 and 2 isolates (7% to 93%). In addition to the 2 groups of RSV isolates, the reaction patterns with the MAbs identified 7 distinct strains, ≥2 distinct epitopes, among group 1 isolates and 4 among group 2 isolates. The number of antigenic variants among RSV isolates makes it possible to use antigenic differences to study patterns of transmission of RSV. The extent of variation among isolates, limits the ability to compare group, or subtype, designations between laboratories.

R25.28

SURFACE GLYCOPROTEINS OF RESPIRATORY SYNCYTIAL VIRUS: THEIR ROLE IN PROTECTION. E.J. Stott[1], L.H. Thomas[1], J. Furze[1], N.J. Ford[1], K.K-Y. Young[2], L.A. Ball[2], & G.W. Wertz[2]. [1] Institute for Animal Disease Research, Compton, Berkshire, UK. [2] Department of Microbiology, University of Alabama, Birmingham, USA.

Recombinant vaccinia viruses expressing attachment glycoprotein (G) or fusion glycoprotein (F) of respiratory syncytial virus (RSV) were used to study the surface expression of RSV proteins. Immunofluorescence with monoclonal antibodies indicated that over 70% of cells expressed F or G antigen within 6 hours of infection with recombinant vaccinia viruses. These cells were lysed by incubation with appropriate antibody and rabbit complement.

Vaccination of mice and cotton rats with recombinant viruses protected the animals against intranasal challenge with RSV. The vaccinia viruses were administered by the intranasal, intraperitoneal or subcutaneous routes. Cattle have also been inoculated with recombinant virus and challenged with a bovine strain of RSV.

R25.29

SEQUENCE ANALYSIS OF THE RINDERPEST VIRUS GLYCOPROTEIN GENES AND COMPARISON TO THE MEASLES VIRUS GLYCOPROTEINS. K. Tsukiyama, M. Sugiyama, Y. Yoshikawa, K. Yamanouchi. Laboratory Animal Research Center, Institute of Medical Science, University of Tokyo, Minato-ku, Tokyo 108, Japan.

We constructed cDNA library from rinderpest virus (RV)-L strain infected Vero cell mRNA, and obtained the full-length cDNA clones of its hemagglutinin (H) or fusion (F) genes.

The nucleotide sequence of RV-H was determined. The gene of RV-H was composed of 1952 nucleotides and contained a single large open reading frame, which was capable of encoding a protein of 609 amino acids with a molecular weight of 68,330 dalton (Da.). The nucleotide and predicted amino acid sequence of RV-H were compared with those of measles virus (MV)-H. The nucleotide sequence and amino acid sequence showed 63.4% and 59.3% homology, respectively. Homology of amino acid sequence was 78.0% in the N-terminus, 49.5% in the middle region, and 58.8% in the C-terminus. The positions of 13 cystein residues of RV-H were completely identical to that of MV-H. Their hydropathy profiles were resembled each other, and one major hydrophobic region with sufficient length for anchor in the membrane was located near the N-terminus.

R25.30

CLONING, EXPRESSION AND SEQUENCE OF THE FUSION (F) PROTEIN FROM HUMAN RESPIRATORY SYNCYTIAL VIRUS (RSV).J.A. Melero, J.A. López, M.Villanueva, B. García-Barreno, C.Peñas and A. Portela. Centro Nacional de Microbiología. Majadahonda. Madrid. Spain.

A cDNA library of RSV genes (Long strain) has been obtained from mRNA of Hep-2 infected cells using previously described strategies and pRSV9 shuttle vector (1).Thus, complete DNA copies of RSV mRNAs are inserted, correctly oriented, under the early promotor of SV40.

Transient expression of the F gene has been attempted, upon transfection of COS cells. Although mRNA was observed by Northern analysis, no production of F protein was detected by a variety of immunochemical techniques. To rule out the presence of mutations which could hamper the synthesis of authentic protein, the cloned gene has been sequenced. Several aminoacid changes were detected as compared with the published sequences of the A2 strain; however, no changes were observed which could explain the failure to produce intact protein. At present, site-directed mutagenesis is being conducted to overcome the problems for expression of the F gene. Sequence results further indicate that genetic variability among RSV strains is very limited.

(1) Portela, A., et al., Virus Res., 4 69 (1985)

R25.31

AMINO ACID SEQUENCING OF THE MAJOR NUCLEOPROTEIN OF EBOLA VIRUS CONFIRMS IT'S GENE LOCATION TO THE 3' END OF THE VIRAL GENOME.....L.H. Elliott, B.P. Holloway, and J.B. McCormick.....Centers for Disease Control, Atlanta, GA 30333, U.S.A.....

Ebola virus is a member of the proposed Filoviridae. It consists of a single piece of negative strand RNA and seven structural proteins. In order to assist in determining the coding assignments of the Ebola genes we began to study the amino acid sequence of purified nucleoprotein (NP), an 82Kd viral structural protein. The NP has a blocked N-terminus requiring treatment with TPCK-trypsin, and subsequent fragment purification on RP-HPLC performed on an Altex C8 column. We have shown that three fragments, one near the N-terminus (resides 15-28), and two centrally placed fragments (residues 254-260 and 390-395) correspond with sequences derived from CDNA clones of the Ebola genome. We conclude from these sequences (and others) that the principal NP of Ebola virus is encoded in the 3' proximal gene, a characteristic of paramyxoviruses and rhabdoviruses. Data will also be presented on the reaction with ebola specific monoclonal antibodies of fragments derived from acid and CNBr cleavages.

R25.32

SEQUENCE ANALYSIS OF THE EBOLA VIRUS GENOME INDICATES THAT IT IS SIMILAR IN ORGANIZATION TO PARAMYXOVIRUSES AND RHABDOVIRUSES. A. Sanchez, M. P. Kiley, B. P. Holloway, J. B. McCormick, D. D. Auperin. Division of Viral Diseases, Centers for Disease Control, Atlanta, Georgia, USA.

Previous studies have demonstrated that Ebola virus mRNA is complementary to virion RNA, thus confirming the negative-strand nature of this virus. Subsequent cloning and sequencing studies have provided information on the first three genes, and have shown the Ebola virus genome to be organized similar to other negative-stranded RNA viruses containing monopartite genomes, namely paramyxo- and rhabdoviruses viruses. At the 3' end of the genome, a short putative leader sequence of 53 bases is followed by the nucleoprotein gene. Genes are delineated by conserved sequences seen at the 3' and 5' ends of each gene, with the later serving as a polyadenylation site during transcription. Intergenic sequences have also been determined. These sequences act as transcriptional signals, and are functionally similar to those seen in paramyxo- and rhabdoviruses.

R25.33

MEMBRANE TRANSLOCATION OF THE HN GLYCOPROTEIN OF NEWCASTLE DISEASE VIRUS. C. Wilson, R. Gilmore, T. Morrison. Departments of Molecular Genetics and Microbiology, and Biochemistry, University of Mass. Medical School, Worcester, MA, USA.

The hemagglutinin-neuraminidase protein (HN) of Newcastle Disease Virus (NDV) most likely contains a bifunctional signal-anchor transmembrane region near the amino terminus. Full length cDNA clones encoding the HN glycoprotein have been constructed and inserted into an SP6 vector. Transcription of the HN cDNA with SP6 polymerase produces mRNA which directs the synthesis of authentic HN protein in a wheat germ cell-free protein synthesis system. Synthesis of HN in the cell-free system supplemented with microsomal membranes (RM) results in the production of a 74 kd HN molecule. We have shown that the 74 kd HN is inserted into microsomal vesicles co-translationally and requires SRP for insertion. Mutagenesis of the cDNA encoding the amino terminus of HN has been used to investigate the role of primary amino acid sequence in the proper membrane insertion of HN. Results of such mutational analysis will be presented.

R25.34

FORMATION OF DISULFIDE LINKED OLIGOMERS OF NDV HN PROTEIN IS NOT REQUIRED FOR INTRACELLULAR TRANSPORT. T. Morrison and L. McGinnes. Department of Molecular Genetics and Microbiology, University of Massachusetts Medical School, Worcester, MA, USA.

The hemagglutinin-neuraminidase (HN) proteins of many paramyxoviruses are in the form of disulfide linked oligomers. The intracellular site of the formation of the disulfide linked oligomers of the HN protein of Newcastle disease virus (NDV), strain AV, was investigated using inhibitors of glycoprotein transport. Carbonyl cyanide M-chlorophenylhydrazone (CCCP) blocked the formation of disulfide-linked oligomers while monensin did not, suggesting that intermolecular disulfide bonds form as the protein moves from the rough endoplasmic reticulum to the medial Golgi membranes. While the formation of the oligomer results in the appearance of one of the five antigenic sites on the protein, not all intracellular HN protein is converted to disulfide linked oligomers. 20-30% of cell surface HN protein is in the form of monomers. Also, the kinetics of insertion into and release from the plasma membrane of the monomer and oligomer forms of the HN protein are identical. Thus formation of disulfide linked oligomers does not facilitate transport of the protein to the cell surface or incorporation of the protein into virions.

R25.35

Amino Acid Substitutions Affecting Cleavage of the Fusion Glycoprotein of Human Respiratory Syncytial Virus. K. Anderson, L. A. Ball, L.E. Dickens and G.W. Wertz. The University of Alabama at Birmingham, Birmingham, AL 35294.

The F protein of Respiratory Syncytial virus is found on the surface of virions and virus-infected cells and is responsible for the cell-cell fusion activity typical of the virus-induced cytopathic effect in cell culture. Two cDNA clones of the F protein of Human Respiratory Syncytial virus strain A2 were expressed in recombinant vaccinia virus vectors. Recombinant vaccinia virus containing RS F cDNA A26 expressed an F protein that comigrated with F_0, the uncleaved glycosylated precursor of the mature form of the F protein. Recombinant vaccinia virus containing RS F cDNA A25 expressed the mature form of the F protein, the disulfide bond linked F_1 and F_2 subunits, in addition to F_0. The complete nucleotide sequence of both cDNA clones were compared to determine the change(s) in the amino acid sequence responsible for the lack of cleavage of the F protein expressed by recombinant vaccinia containing RS F cDNA A26. Five nucleotide substitutions resulting in four amino acid differences between the two F proteins were observed. Experiments are currently in progress to obtain recombinant F proteins by exchange of restriction fragments between the two cDNA clones and by site directed mutagenesis to determine the amino acid change(s) responsible for the lack of cleavage of the F protein.

R25.36

THE PURIFICATION OF FOUR RESPIRATORY SYNCYTIAL VIRUS PROTEINS AND THEIR PERFORMANCE AS PROTECTIVE AGENTS AGAINST INFECTION IN MICE. E.G. Routledge, M.M. Willcocks, A.C.R. Samson, L.A. Morgan, R. Scott, G.L. Toms. Departments of Virology & Genetics, University of Newcastle upon Tyne, United Kingdom.

The fusion (F), large glycoprotein (G), phosphoprotein (P) and 22K protein of RS virus A2 strain were purified by a combination of immunoaffinity adsorption and preparative SDS-PAGE. All four proteins elicited serum antibody in mice after repeated inoculation in Freund's adjuvant, although the magnitude of the response as measured by ELISA varied from mouse to mouse. The F protein generated neutralizing antibodies in only 50% of the mice determined to be seropositive by ELISA. The G protein, however, induced neutralizing antibodies which correlated well with ELISA titres. No neutralizing activity was detected in mice immunized with the P and 22K proteins. Mice immunized with the F and G proteins were found to be protected against subsequent RS virus infection, even in those cases where no neutralizing antibody could be detected. Mice inoculated with the P and 22K proteins were not protected.

R25.37

ANTIGENICITY OF MEASLES VIRUS HEMAGGLUTININ STUDIED BY SYNTHETIC PEPTIDES. M.I. Mäkelä, G. Lund, R.H. Smith and A. Salmi. Department of Medical Microbiology and Infectious Diseases, University of Alberta, Edmonton, Alberta, Canada.

Hemagglutinin (HA) is the major surface glycoprotein of measles virus against which the majority of neutralizing antibodies are directed. To characterize the antigenicity of HA at a molecular level we synthesized five peptides corresponding to its most likely antigenic sites exposed at the surface. The prediction of these sites was done by an algorithm using the known sequence of the MV hemagglutinin gene. Antisera against virions, purified HA and monoclonal antibodies (MAb) against HA were studied in a direct EIA for binding to these peptides Three of the peptides were recognized by anti-HA serum and four by anti-virus serum indicating that these peptides are exposed at the surface of HA. One of the synthetic peptides was strongly recognized by one MAb. Peptides conjugated to keyhole limpet hemocyanin were injected into rabbits to produce antisera. These sera were tested in direct EIA for binding to purified virions, a lysate prepared from measles virus infected cells and to homologous peptides conjugated with BSA . All five anti-peptide antisera had high titers against the homologous peptides and recognized purified virus preparations whereas only three of them bound to the cell lysate antigen. Three of the sera bound to the HA polypeptide in immunoprecipitation and western blotting tests. Our preliminary results indicate that one of these peptides is recognized by murine T cells in a proliferation assay. These results define at least one immunodominant region of MV hemagglutinin and demonstrates the feasibility of using synthetic peptides as probes for antigenicity of viral proteins. THIS STUDY WAS SUPPORTED BY THE ALBERTA HERITAGE FOUNDATION FOR MEDICAL RESEARCH.

R25.38

MEASLES VIRUS FROM A LONG-TERM PERSISTENT INFECTION, IN CONTRAST WITH THE CYTOCIDAL PARENTAL VIRUS, ESTABLISH AN IMMEDIATE PERSISTENCY IN THE ORIGINAL CELL LINE. R. Fernandez-Muñoz, M.L. Celma. Unidad de Virología, Hospital "Ramon y Cajal" Instituto Nacional de la Salud, Madrid, Spain.

We have established a persistent infection with Measles Edmonston in the human lymphoblastoid T cell line MOLT4, MOMP1, that is in continuous culture for five years. MOMP1 releases infectious virus with a yield 100fold less than the peak of acute infection, that remains constant so does the number of infectious centers. Although the ratio of p.f.u. to viral particles is lower in MOMP1, no evidence of defective interfering particles was obtained. (^{32}P) labelled nucleocapsid RNA sedimented as a 52S peak, and T$_1$RNAse fingerprints showed minor sequence changes from the parental virus. The virus released by MOMP1, in contrast with the parental one, which produced a lytic infection with extensive cell fusion in MOLT4 cells, readily establishes a persistent infection in this cell without significant cell viability loss, nor cell fusion. This suggests that the viral modification suffices to mantain the state of persistency without requiring a co-evolution of the host cell during the infection, as has been reported in other viral systems.

R25.39

ANTIGENIC ANALYSIS OF NEWCASTLE DISEASE VIRUS MEMBRANE PROTEINS. A.J. Della-Porta and E. Hansson. CSIRO Australian Animal Health Laboratory, Geelong, Victoria, Australia.

Newcastle disease virus (NDV) is the cause of serious losses in the poultry industries of most countries except Australia and New Zealand. NDV vaccines are the most extensively used of any viral vaccine and mainly utilise live low virulence NDV strains. In order to define the immunological role of NDV proteins and to identify important antigenic epitopes, studies are being carried out with purified antigens, in as native a state as possible, and monoclonal antibodies. NDV contains four membrane proteins; a dimer of the hemagglutinin - neuraminidase (HN) glycoprotein; a disulfide linked fusion glycoprotein consisting of two fusion protein subunits, F_1 and F_2; and the highly hydrophobic matrix (M) protein. A wide variety of mild solubilization techniques have been investigated to extract these proteins from the viral membrane. The use of the ionic detergent sodium deoxycholate (0.1%) enabled efficient extraction of the glycoproteins HN and F_1-F_2. The use of a Triton X-100 (1%)/sodium deoxycholate (0.5%) mixture in the presence of 1M potassium chloride enabled efficient extraction of HN and M, but resulted in poor yields of F_1-F_2. Proteins solubilized by both procedures were highly immunogenic and stimulated the production of virus-neutralizing antibodies. The membrane proteins were purified by a variety of mild techniques, including affinity chromatography, and their antigenic epitopes are being mapped.

R25.40

RESTRICTED SYNTHESIS OF MEASLES VIRUS MATRIX PROTEIN IN A LONG-TERM PERSISTENT INFECTION OF A HUMAN LYMPHOBLASTOID CELL LINE. M.L. Celma, R. Fernandez-Muñoz. Unidad de Virología, Hospital "Ramon y Cajal", Instituto Nacional de la Salud, Madrid, Spain.

We have established a persistent infection, MOMP1, in the cell line MOLT4, with Measles Edmonston, that is in continuous culture for more than five years. In contrast with the acute infection, where cytopathic effect consist in extensive cell fusion, in MOMP1 only very scarce polykaryons are observed. All major viral proteins are present without apparent changes in M.W. The fusion protein Fo is glycosilated and cleaved with similar efficiencies than in the acute infection. However there was a diminished accumulation of matrix (M), and haemagglutinin (H) proteins. Pulse-chase experiments showed that in MOMP1 there was a reduction in the rate of synthesis of M protein, which did not present a preferential rate of degradation. The messenger RNAs for M and other viral proteins accumulated at a similar relative rate in the acute and persistent infections.
Conclusions: In MOMP1 there is a restriction in the expression of M protein that is not explained by its rapid degradation, as reported for an SSPE cell line, rather it seems due to a less efficient translation, as reported for SSPE brain.

R25.41

AN ATTEMPT TO CLONE THE COMPLETE GENOMES OF MEASLES VIRUS MESSENGER RNA. W. Luo and D.L.J. Tyrrell, Department of Medical Microbiology and Infectious Diseases, Faculty of Medicine, University of Alberta, Edmonton, Alberta, Canada.

The mRNAs of the Lec strain of measles virus were extracted from infected Vero cells on oligo dT-cellulose columns. The first complementary DNA strand was synthesized using oligo dT priming and AMV reverse transcriptase. The second DNA strand was synthesized using RNas H and DNA polymerase I.

We demonstrated cDNA bands (1.5 Kb to 5.5 Kb) on gel electrophoresis and autoradiography. The cDNA fragments were cloned into plasmid vectors. Positive clones were selected by antibiotic sensitivity or on X-gal plates. These clones were characterized by hybridization with previously identified cDNA fragments from measles virus. The size of the cloned fragments was determined by restriction endonuclease cleavage. These clones will be expressed in eucaryotic cells in an attempt to produce functional measles virus proteins.

R25.42

CHARACTERISTICS OF AN ALLERGEN FREE MEASLES-MUMPS-RUBELLA VACCINE.
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Swiss Serum and Vaccine Institute Berne and University Childrens Hospital, Basle, Switzerland.

Anaphylactic reactions due to measles-mumps-rubella vaccinations are usually attributed to avian proteins or neomycin which are contained in such vaccines.

A new vaccine produced in human diploid cells without addition of antibiotics has the following components:

1) Edmonston-Zagreb (EZ19) measles virus, an Edmonston Enders strain, further attenuated and adapted to HDC by D. Ikic.
2) Rubini mumps virus, attenuated and adapted to HDC by R. Glück.
3) RA 27/3 rubella virus attenuated and adapted to HDC by S.A. Plotkin.
4) A stabilizer containing no substances of animal origin. This guarantees stability of all components for at least 7 days at 37°C.

The overall seroconversion rate as evaluated in 290 seronegative children aged between 15 months and 16 years was 95-100%. The protocolled tolerance showed optimal results.

R25.43

ANTIGENIC COMPARISON OF PARAINFLUENZA VIRUS TYPE 3 ISOLATES WITH MONOCLONAL ANTIBODIES. DISTINCT DIFFERENCES BETWEEN HUMAN AND BOVINE STRAINS.

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Department of Virology, Karolinska Institute, School of medicine, c/o SBL, S-105 21 Stockholm, Department of Virology, National Bacteriological Laboratory, S-105 21 Stockholm, Sweden.

The antigenic characteristics of eight human and three bovine strains of parainfluenza virus type 3 were analyzed. The strains were compared by use of 52 monoclonal antibodies against 5, 2, 6 and 6 epitopes of the hemagglutinin-neuraminidase (HN), fusion (F), nucleocapsid (NP) and matrix (M) viral proteins respectively, employing radioimmuno precipitation and immunofluorescence assays.

The human strains, seven of which were isolated over a time span of six years at different geographical locations and the eighth one representing an older prototype strain, showed very little antigenic variation. Extensive differences were detected in all four proteins examined between the human strains and the three strains of bovine origin. Bovine strains were less effectively neutralized than the prototype strain with a series of monoclonal antibodies against the HN protein.

R25.44

PROTECTIVE EFFECTS OF MONOCLONAL ANTIBODIES AGAINST PARAINFLUENZA VIRUS TYPE 3 INDUCED ENCEPHALITIS IN HAMSTER.

R. Rydbeck, A. Löve, E. Norrby.
Department of Virology, Karolinska Institute, c/o SBL, S-105 21 Stockholm, Sweden.

A collection of monoclonal antibodies (Mabs) against the hemagglutinin-neuraminidase (HN) and fusion (F) glycoproteins of parainfluenza virus type 3 was used for passive immunization of infected newborn hamsters. No individual antibody conferred total protection against disease, however a significant degree of protection was obtained with Mabs against both the HN and F viral proteins. Mabs against different epitopes exhibited a different degree of protection. A correlation with high, hemagglutination inhibition and neutralization in vitro could be seen.

R25.45

SEQUENCE DETERMINATION OF THE MEASLES VIRUS L GENE AND ANALYSIS OF THE GENOME 5' END REGION. Benjamin Blumberg*, Joel Silverman, Barry Schanzer, Eytan Young, Matthew Levy, Joan Crowley, Stuart Cook and Peter Dowling. Neurovirology Laboratory, East Orange VA Medical Center, East Orange, NJ 07019, and Depts. of Neurosciences and *Microbiology, UMDNJ-New Jersey Medical School, 100 Bergen St., Newark, NJ 07103, USA.

We have constructed a measles virus (MV) cDNA library encompassing the entire MV genome, and determined the nucleotide sequence of the L gene using 50 of more than 200 identified L clones. The L gene is 6641 nucleotides in length, and contains a single long ORF that could code for a protein of 244,376 Kdal. Comparison of the predicted MV L protein structure with that of Sendai virus shows a degree of homology so high that there can be no doubt that these paramyxoviruses evolved from a common ancestor. We also determined the nucleotide sequence of the MV 5' end region from cloned DNA and by primer extension on MV genomic RNA. In combination with previously published sequence data for the MV N, P/C, M, F and H gene regions, our data completes the sequence determination of the MV genome, which is 15,894 nucleotides in length. The last 11 nucleotides are complementary to the genome 3' end sequence, and the antigenome could code for a putative (-) leader RNA of about 35 nucleotides. Curiously, however, no leader RNAs were detected in extracts from MV infected cells.

R26.1

GENETIC HETEROGENEITY IN THE LAMBDA FAMILY OF TEMPERATE COLIPHAGES. Alice P.W. Poon. Department of Botany, University of Hong Kong, Hong Kong.

26 temperate coliphages either present as cell-free virions in sewage or as prophage in naturally occurring strains of E.coli were characterized. Majority of those present as cell-free virions adsorbed to strains C and K12 of E.coli but not to their tonA mutants (host range T5 or HRT5). A large proportion of those present as prophages failed to adsorb to K12 bacteria but did adsorb to E.coli C and its tonA mutants (C-specific). Both groups of prophages were inducible by UV. Out of 13 HRT5 isolates tested, 8 were found to integrate near the proAB locus and showed the restricted transduction of $proA^-/B^-$ mutants to Pro^+. Two of the HRT5 phages were found to integrate near the his locus and transduced the his^- mutants. None of the 13 HRT5 phages was found to integrate at att^{λ}. The C-specific phages showed more diverse integrational specificities. Out of 13 tested only 2 showed integration near proAB. All were tested to see if they integrate at some of the other att loci and none was found to utilize att^{λ}(17), att^{HRT39}(44), or att^{HTT}(22). A number of representatives of each host range class were crossed with λ and recombinants possessing the immunity specificity of one parental phage and host range of the other were readily detectable. Some of the C-specific phages can convert the K cells rendering them sensitive to heteroimmune C-specific phages.

R26.2

PHYLOGENETIC STUDIES ON RNA COLIPHAGES. K. Furuse[1], Y. Inokuchi[2], A. Hirashima[3]. [1]Department of Microbiology, School of Medicine, Tokai University, Isehara-shi, Japan, [2]Department of Molecular Biology, School of Medicine, Keio University, Shinjuku-ku, Tokyo, Japan, [3]Yakult Central Institute for Microbiological Research, Kunitachi, Tokyo, Japan.

According to the serological and several physicochemical properties, RNA coliphages can be classified into four groups (I through IV). On the basis of the above-mentioned classification, group IV phages were found to exhibit more diversity in several respects, such as serological characters, the molecular weights of the viron proteins, electrophoretic mobilities of the phage particles, and the primary structure of the 3'-terminal region of the phage RNAs. To clarify the phylogenetic relationships among the four RNA phage groups, it would be necessary to compare not only the structural aspects of the phage particles but also the functional ones such as degree of genetic complementation and template activity of the RNA replicase. From these experiments, some phages of group IV exhibited intermediate characteristics in inter-group complementation tests and in vitro RNA synthesizing system. This biological and physicochemical diversity and the intermediate character exhibited by the group IV phages may have some significance in the phylogenetic relationships among the four groups of RNA phages. Taking into consideration the known natures of virus RNA, the author discusses the mechanism of diversification of RNA phages.

R26.3

CHARACTERIZATION OF THE GENOME OF THE BACTERIOPHAGE Ø PLS 27. B. J. Allan, E. B. Carstens and A. M. Kropinski. Department of Microbiology and Immunology, Faculty of Medicine, Queen's University, Kingston, Ontario, Canada.

Phage Ø PLS 27, a podovirus that infects Pseudomonas aeruginosa, appears phylogenetically related to coliphage T7. A restriction map of the linear-double stranded DNA genome of Ø PLS 27 was produced using single and double enzyme digestion. By summation of the sizes of the restriction fragments the genome was found to be 42.5 kb. Southern blot analysis of the restriction fragments from the genome were carried out using a 1.3 kb end fragment (Hpa I-B) as the probe. Terminal redundancy of the genome was observed. Both ends of the genome have been cloned to facilitate analysis of the terminal redundancy. An end fragment (800 bp) of the phage genome, exhibiting promoter activity in E. coli, was cloned using the promoter selection vector pKK232-8. From the sequence analysis a putative promoter sequence, related to that found in the TOL plasmid pWW74, was identified. The end fragment also contained 12 direct repeats based on the sequence CCTGGT. Four long repeats of GCCTTACCTCTTTTGC were also observed. The significance of these repetitive structure is not known.

R26.4

CHARACTERIZATION OF THE T-ODD COLIPHAGE BA14, AND THE RELATION OF ITS EARLY REGION WITH PHAGES T7 AND T3.

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Coliphage BA14 belongs to the T-odd phage family, according to biochemical, genetic and serological evidence [Hertens, H. and Hausmann, R. (1982) J. Gen. Virol. 62, 331-341]. These data indicate that BA14 is as different from T7 and T3 as they are from each other, and can thus be considered a representative of a distinct T-odd phage group. We have been engaged in characterizing in greater detail the genome of phage BA14, and in mapping the early region genes and characterizing early gene products through in vivo and in vitro assays. A genomic restriction map has been constructed, using at least nine restriction endonuclease specificities. Based on these results, the functional relationships of the BA14 early gene products, in particular the serine/threonine-specific protein kinase activity, to those of phages T3 and T7 will be presented.

R26.5

ON THE USEFULNESS OF PHAGE TAXONOMY WITH PARTICULAR REFERENCE TO VIBRIO PHAGES.
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The importance and usefulness of viral (phage) taxonomy have been highlighted with special reference to vibrio phages. Both fundamental and applied or practical aspects of the use of virus taxonomy have been elaborated. Classification of vibrio phages into four families (Myoviridae, Styloviridae, Podoviridae, and Inoviridae) and 18 species have been illustrated. Practical uses of vibrio phage classification have been elaborated with particular reference to the findings related to (a) phage typing of cholera vibrios, (b) differentiation of cholera and el tor biotypes, (c) rapid disappearence of the classical biotype and its replacement by the el tor biotype in India and most parts of world, (d) detection of the four independent foci harbouring different phage types of V. cholerae in the Indo-Pakistan subcontinent and (e) detection of the mode or modes of entry and spread of the disease caused by the el tor biotypes in this subcontinent. The need for the development of a universal classification scheme of viruses (phages) has been emphasized.

R26.6

COMPARISON OF BREVIBACTERIUM FLAVUM BACTERIOPHAGES. V.Z.Akhverdian, S.G.Haroutunian, E.A.Khrenova, B.P.Karabekov, V.N.Krylov. Institute for Genetics of Microorganisms, Moscow, and Research Institute for Aminoacids, Yerevan, U.S.S.R.

The comparison of 10 independent isolates of phages active on Brevibacterium flavum have been done. All bacteriophages are morphologically identical: Bradley's B-type, head diameter is 60 nm, length of tail is 310 nm. Molecular sizes of phage genomes are 44-47 kb. According to results of DNA homology studies the bacteriophages have been distributed into 3 subgroups: ØB subgroup (7 phages), EØ subgroup (2 phages) and HØ subgroup (1 phage). Phages belonging to the same subgroup are serologically related, and their genomes have more than 90% DNA homology. The level of DNA homology between phages of ØB and EØ subgroups is varying from 0 to 5% in different combinations; for phages of EØ and HØ subgroups the DNA homology is about 1-2%. No DNA homology have been found in pairs of ØB and HØ phages. Thus, although the study of DNA homology is very important in phage classifications, sometimes it have no crucial significance in conclusion on relatedness of bacteriophages even for phages active on the same bacterial species.

R26.7

MORPHOLOGY OF PSEUDOMONAS AERUGINOSA TYPING PHAGES. H.-W. Ackermann, J.-P. Vieu. Department of Microbiology, Faculty of Medicine, Laval University, Quebec, Canada; Service des Entérobactéries, Institut Pasteur, Paris, France.

Eighteen phages of Lindberg's typing set were stained with uranyl acetate and phosphotungstate and were examined in a Philips EM 300 electron microscope. Phages were tailed and belonged to 6 morphotypes. Heads were icosahedral or elongated. The phages belonged to the Myo-, Sipho-, and Podoviridae families. Most of them represented well-known phage species. Ten phages were myoviruses, belonging to species φKZ (phages 21 and 68) and PB-1 (16, 24, 44, 109, 352, 1214, F8, M4). Phages of the φKZ type are very large, having heads of 125 nm in diameter and 180 nm long tails, provided with large collars and a sheath-like fibrous network. Siphoviruses were represented by 4 phages. Three of them had isometric heads of 57 nm in width and tails that were either 180 nm (73 and F7) or 133 nm long (F10). Phages 73 and F7 seem to represent a new species; F10 can be related to species D3. Phage M6 had an elongated head of 77 x 50 nm and a tail of 152 nm in length; it is the type virus of a species of its own. Podoviruses included 4 T7-like phages (2, 7, 31, 119x) with 61 nm-wide heads and short, tapering tails. They were attributed to species gh-1. Results are in good agreement with serological data.

R27.1

ISOLATION OF CLONAL DERIVATIVES OF RD-114 CELLS WHICH RESPOND TO INTERFERONS DIFFERENTLY. R. Kumar, R. Tiwari, G.C. Sen. Molecular Biology Program, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, N. Y. 10021, U.S.A.

RD-114, a human rhabdomyosarcoma line, does not exhibit the full repertoire of responses to interferons (IFN). For example, in these cells replication of retroviruses but not of vesicular stomatitis virus (VSV) or of encephalomyocarditis virus (EMCV) is inhibited by IFN. Similarly induction of some of the IFN-inducible mRNAs but not of others is poor in these cells. Recently we have isolated several clonal derivatives of this cell line. In eight of these, we systematically examined the patterns of inhibition of replication of VSV, EMCV and retroviruses and of induction of 2-5(A) synthetase mRNA in response to IFN-α and IFN-γ. We observed that there was a great diversity in the spectra of responses among these clones. For example, EMCV replication was strongly inhibited in clone A2 but not in clone B6 whereas VSV replication was strongly inhibited in clone B1 but not in clone A3. Our analyses also revealed that induction of 2-5(A) synthetase mRNA cannot be directly corelated with inhibition of EMCV replication. The cell clones described here will be useful both for understanding the mechanism of IFN-mediated gene induction as well as for elucidating the IFN-mediated pathways involved in inhibition of replication of various viruses. (Supported by grants from the National Institutes of Health).

R27.2

ACTIVATION OF NATURAL KILLER CELLS IN NEWBORN PIGLETS BY INTERFERON INDUCTION. C.E. Lesnick, J.B. Derbyshire. Department of Veterinary Microbiology and Immunology, University of Guelph, Guelph, Canada.

The treatment of 17 newborn piglets with 0.5 mg/kg of polyinosinic polycytidylic acid complexed with poly-L-lysine and carboxymethylcellulose (poly ICLC) induced a transient circulating interferon (IFN) response which peaked at 6 hours post-induction (PI). Activation of natural killer (NK) cells among the peripheral blood lymphocytes of the treated piglets peaked at 24 hours PI at a level similar to that found in normal weaned piglets, and subsequently declined to the level found in untreated controls by 7 days PI. The NK activity in the treated and control piglets then increased at a similar rate, reaching the levels observed in weaned piglets by 16 days of age. When 5 poly ICLC-treated newborn piglets and 5 untreated controls were exposed to infection with transmissible gastroenteritis (TGE) virus, a porcine enteric coronavirus, there was a delay in onset of clinical signs in the treated piglets, although the subsequent course of the disease was similar to that in the controls. It was concluded that IFN induction in newborn piglets is associated with NK activation and some increase in resistance to TGE.

R27.3

MECHANISM OF GENE INDUCTION BY INTERFERONS. J. Chebath, M. Vigneron, D. Peretz and P. Benech. Department of Virology, Weizmann Institute of Science, Rehovot 76100, Israel.

A human gene encoding two forms of (2'-5') oligo A synthetase (E-gene) was used to study the mechanism of gene activation by Interferons (IFNs). The 0.83 kb fragment of E-gene 5'-flanking sequences and serial 5' and 3' deletions of this fragment, were studied for their ability to drive the transient expression of the chloramphenicol acetyltransferase (CAT) gene in various human cells, and to respond to IFN treatment of the transfected cells. These experiments allowed to delimit a 72 bp fragment which renders CAT expression inducible from 10 to 50 times by IFN-α or β, but not by IFN-γ suggesting that the latter may act by another mechanism than IFN-α-β. The interferon responsive sequence of the E-gene (E-IRS) is active on its own promoter as well as on the Herpes virus thymidine kinase promoter. The E-IRS is located immediately upstream to the 10 main transcriptional start sites of the E-gene, which are spread over 50 bp and not preceded by a TATA box. The same RNA start sites are activated by IFN in the chimeric CAT constructs during transient expression. The E-IRS contains a long dyad symetry palindrome. Internal deletions of the E-IRS reveal that it is composed of a constitutive transcriptional element followed by a regulatory element required for activation after IFN treatment. The regulatory element could be binding a repressor protein in cells untreated by IFN.

R27.4

PROTECTIVE EFFECT OF RECOMBINANT MURINE INTERFERON BETA AGAINST MOUSE HEPATITIS VIRUS INFECTION. R. Mori, H. Minagawa, A. Takenaka, S. Mohri. Department of Virology and Laboratory of Animal Experiments, School of Medicine, Kyushu University Fukuoka 812, Japan

Recombinant murine interferon beta (IFN) protected susceptible C57BL/6 mice against lethal mouse hepatitis virus type 2 (MHV-2) infection. Mice were infected intraperitoneally with 100 pfu (more than 10 LD50) of MHV-2. High doses (8,000 IU per day or higher doses) of IFN given daily for 9 days from 21 h before infection completely protected mice against death and severe hepatitis. IFN given from 3 h after infection lengthened the survival time, but IFN treatment beginning 24 h after infection was ineffective.

The survival rate was dose-dependent, and the 50% effective dose of IFN for survival was 1,780 IU per day. IFN pretreatment inhibited virus growth completely in the brain and moderately in the liver and spleen.

IFN also protected beige mice and cyclophosphamide-treated mice, suggesting that activation of natural killer cells or immune responses by IFN was not critical for protection. (Recombinant murine interferon beta was provided by Basic Research Laboratories, Toray Industries Inc., Tokyo)

R27.5

PRODUCTION OF ACTIVE ppp2'-5'$(Ap)_n$A OLIGOMERS USING A HUMAN 2'-5'-OLIGO(A) SYNTHETASE MADE IN *E. coli*. Y. Mory, B. Vaks and J. Chebath. The Dept. of Virology, Weizmann Institute of Science, Rehovot 76100 and InterYeda Ltd., Kiryat Weizmann, Ness-Ziona, Israel.

The 2'-5'-oligo(A) synthetase is an interferon-induced enzyme ds-RNA activated, producing 2'-5' linked oligomers of ATP (ppp2'-5'$(Ap)_n$A which activates a latent ribonuclease and inhibits protein synthesis. Oligomers modified to allow their penetration into cells or improve their stability have been shown to inhibit viral growth and/or cell growth for several days after their addition. One of the main limitations in further studying the biological functions of this potential therapeutic aspect is the low amounts obtained by incubation of mammalian cell extracts treated by IFN. We have cloned in an *E. coli* expression vector the cDNA of the human 40 KDa 2'-5'-oligo(A) synthetase. The *E. coli* enzyme purified to near homogeneity and immobilized on poly (I:C) agarose produces, per ml of poly (I:C), 10^5 times more 2'-5'A oligomers than the most active cellular extracts. Moreover, the immobilized enzyme remains stable for several months at 4°C. Some other catalytic properties of the 2'-5' oligo(A) synthetase, such as 2' adenylation of NAD and Ap_4A or the use of other nucleotides as substrates opens the possibility to prepare new molecules of which the potential biological effects have to be explored.

R27.6

RAPID ENHANCEMENT OF β_2 INTERFERON/B CELL DIFFERENTIATION FACTOR BSF-2 GENE EXPRESSION BY DIACYLGLYCEROLS: ANTIVIRAL EFFECTS IN HUMAN FIBROBLASTS. P. B. Sehgal, Z. Walther and I. Tamm. The Rockefeller University, New York, NY 10021 USA.

The expression in human fibroblasts of the β_2 interferon (IFN-β_2) gene, which is now recognized to be identical to the B-cell differentiation factor BSF-2 gene, is enhanced by several cytokines that affect cell growth (tumor necrosis factor, interleukin-1, platelet-derived growth factor and β_1 interferon). We have examined the possibility that IFN-β_2 gene expression is regulated through activation of the protein kinase C pathway. The synthetic diacylglycerol agonists 1,2-dioctanoylglycerol (diC_8) and 1-oleoyl-2-acetylglycerol (OAG) strongly enhance IFN-β_2 gene expression in human fibroblasts (FS-4 strain). An increase in IFN-β_2 gene transcription is detected within 5 minutes of addition of diC_8 and the rate of transcription is near-maximal by 30 minutes. The diacylglycerol-induced increase in mRNA levels is specific to IFN-β_2 in that IFN-β_1 mRNA is not detected in diC_8- or OAG-treated FS-4 cells. FS-4 cells treated with diC_8 are protected from the cytopathic effect of vesicular stomatitis virus; this protection can be blocked by polyclonal or monoclonal antibodies that neutralize IFN-β indicating that the antiviral effect observed is due to the secretion of IFN-β_2 by the diC_8-treated fibroblasts. These results show that protein kinase C-activating agents rapidly increase the expression of the IFN-β_2 gene in human fibroblasts.

R27.7

CHARACTERIZATION OF THE VACCINIA-ASSOCIATED INHIBITOR OF THE PROTEIN KINASE INDUCED BY INTERFERON. P. Whitaker-Dowling, J. Wu, J. S. Youngner. Department of Microbiology, Biochemistry and Molecular Biology, School of Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania USA.

Vaccinia virus rescues vesicular stomatitis virus from the inhibitory effects of interferon in mouse L cells. This rescue requires early vaccinia gene expression, occurs at the level of protein synthesis, and is linked to the production of a vaccinia-associated inhibitor of the interferon-induced protein kinase. This specific kinase inhibitory factor (SKIF) is detected in cell extracts within 90 minutes of infection by vaccinia virus but is not found in vaccinia virions. The inhibition by SKIF is reversible, and SKIF is neither a phosphatase, an RNase nor a protease. It is nondialysable, trichloroacetic acid precipitable and sediments in sucrose at 5.9S suggesting that the inhibitor is a macromolecule. Evidence has been obtained that SKIF is not an RNA molecule since it is insensitive to RNases and to treatment with 0.2M HCl and is not extracted by phenol. SKIF activity bands in CsCl gradients at a density of 1.4gr/cc, a density characteristic of protein.

R27.8

LOCAL INTERFERON AND ENHANCED 2,5A SYNTHETASE ACTIVITY IN FEMALE GUINEA PIGS FOLLOWING GENITAL HERPES SIMPLEX VIRUS INFECTION. H. E. Renis, B. A. Court, R. D. Hamilton, and M. F. Leach, Cancer & Viral Diseases Research, The Upjohn Company, Kalamazoo, MI 49001. USA

Guinea pigs (gp), inoculated intravaginally with 5 x 10^4 PFU HSV-2, were swabbed for virus titers in vaginal secretions and at intervals, were sacrificed and the interferon (IFN) and 2,5A synthetase (2,5A-S) measured in vaginal homogenates. The HSV-2 titers in vaginal swabs were greatest on day 1, then decreased and were cleared by day 9. IFN assayed by VSV plaque reduction on gp kidney cells, was detected in 50% and 67% of homogenates on days 1 and 3, respectively, after inoculation, but not thereafter. Peak 2,5A-S activity (5-fold greater than non-infected group) was observed on day 3 in vaginal homogenates. The activity declined thereafter, and by day 9 was nearly that of the non-infected group. Xanthine oxidase, an enzyme reported to be increased in tissues of mice after INF injection increased about 4-fold during the 9 days following infection, and remained elevated in vaginal homogenates during an additional 5 days. IFN was not detected in serum and only in low titer in a few spleen homogenates during the 14 day observation period suggesting that the IFN response in this model is restricted to the local site of infection.

R27.9

MONENSIN AND CCCP ENHANCE THE EFFICIENCY OF DETECTION OF IFN-PRODUCING CELL IN HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS. C.L.Y. Lee*, S.H.S. Lee*, K.R. Rozee* and F.T. Jay**. Department of Microbiology*, Dalhousie University, Halifax, N.S. and Department of Medical Microbiology**, University of Manitoba, Winnipeg, Man., Canada.

An efficient indirect immunofluorescence (IF) method was established for the detection of IFNα- and IFNγ-producing cells in peripheral blood mononuclear cells. Sendai virus and SEB or PHA were used as inducers for IFNα and IFNγ respectively. Using specific mouse monoclonal antibodies, we visualized a temporal intracytoplasmic distribution of IFN. IFN first appeared as discrete polar staining; this was followed by the presence of bright and diffuse cytoplasmic fluorescence as time progressed.

Monensin and carboxylcyanide m-chlorophenyl-hydrazone (CCCP) are ionophores known to interrupt subcellular transport and terminal glycosylation of secretory proteins. When monensin or CCCP was added at various times to the induced cultures, an increase in the number of IFN-producing cells was observed. Also a greater than 10-fold enhancement in the sensitivity of immunostaining was demonstrated in the treated preparations. This enhanced sensitivity will be useful in the demonstration of suboptimal level of IFN and at the same time reduce the quantity of primary antibody used in IF staining.

R27.10

EFFECT OF INTERFERONS ON HERPES SIMPLEX REPLICATION. P.R. De Stasio, D. Iannello, and M.W. Taylor. Department of Biology, Indiana University, Bloomington, IN 47405, U.S.A.

Interferons have been shown to inhibit the replication of HSV-1 and HSV-2, and are being used clinically. Utilizing HSV-1 genomic probes of the α, β, and γ subgroups, we have examined the transcription of these genes following treatment of cells which 100 units ml of interfecons. When WISH cells are infected with HSV-2 in the presence of alpha, beta, or gamma interferon, and specific mRNA measured by dot blot analysis, at 4 and 8 hours post infection, there was little effect on transcription of α and γ messages, but there was an enhancement of β mRNA synthesis. Utilizing VERO cells and HSV-1 (m.o.i. = 100), there was a 2 hour delay in the synthesis of α mRNA and γ mRNAs in interferon gamma treated cells. These results indicate that the mechanism of action of interferons may differ depending on the cell-virus combination.

R27.11

A COMPARISON OF THE ANTIVIRAL ACTIVITY OF LYMPHOTOXIN (TNF-β) AND INTERFERONS. G. Feng and M.W. Taylor, Department of Biology, Indiana University, Bloomington, IN 47405, U.S.A.

There has been much controversy surrounding the possible anti-viral activity of tumor necrosis factor. Data has been presented indicating an indirect antiviral effect of TNF-α, in which interferon β_2 is induced by the lymphokine. We show that human Hep-2 cells are protected from VSV and EMC infection by recombinant lymphotoxin (TNF-β), whereas WISH cells, although protected by interferons under the same conditions are not protected by lymphotoxin. This lymphotoxin protection is not abolished by antibodies to IFN-β, indicating that the mechanism of protection does not involve the induction of IFN-β_2. The lymphotoxin protection is partially abolished by anti-serum (polyclonal) to interferon γ, but not by anti-serum to interferon α. Whether this is due to some cross reactivity between lymphotoxin and IFN-γ, or due to contaminating antibodies in the serum is under investigation.

R27.12

EFFECT OF RECOMBINANT MURINE INTERFERON-BETA ON CYTOMEGALOVIRUS INFECTION IN MICE. Y. Minamishima, M. Okada. Department of Microbiology, Miyazaki Medical College, Kiyotake, Miyazaki, Japan.

Antiviral effect of recombinant murine interferon-β (rMuIFN-β) against murine cytomegalovirus (MCMV) infection was studied in normal and immunodeficient mice.

A single intraperitoneal (ip) injection of 2.0×10^6 u of rMuIFN-β was effective against ip inoculation of 1.0×10^6 PFU of salivary gland-passaged, virulent MCMV, when it was administered before the challenge. Five successive administrations of 1.0×10^6 u of rMuIFN-β before and after the challenge protected mice from otherwise lethal infection by MCMV. The efficacy was evaluated by an increase in LD50, a decrease in mortality and prolongation of survival time, and a decrease in virus titers replicated in the target organs. The effect of rMuIFN-β was also confirmed by elevated serum 2',5'-oligoadenylate synthetase activity and other laboratory findings. The protective effect of rMuIFN-β was based mainly on augmentation and maintenance of natural killer (NK) cell activity which was otherwise impaired by MCMV infection. However, although less effective than in normal mice, certain protection by rMuIFN-β was observed in NK cell-deficient beige mutant mice.

Thus, the in vivo effect of rMuIFN-β against MCMV seems to be dual; i.e., virostatic effect of rMuIFN-β itself and NK cell-mediated cytotoxic effect on MCMV-infected cells.

R27.13

INTERFERON SENSITIVITY OF HVJ (SENDAI VIRUS) RESISTANT CELLS.
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Altered baby hamster kidney (BHK-R) cells which resist the HVJ (Sendai virus) infection have been passaged in the continuous presence of egg-grown HVJ. BHK-R cells have also been shown to be refractory to the antiviral action of both type I and II hamster interferons.

When confluent monolayers of BHK-R cells were incubated in virus-free maintenace medium under the conditions of no cell division, they still preserved the resistance to HVJ, but gradually became sensitive to the interferon action, and the subsequent superinfection with egg-HVJ or BHK-grown trypsin-treated HVJ restored the cells again to the interferon resistant state. BHK-grown HVJ without trypsin treatment failed to do it. Ultraviolet-irradiated egg-HVJ which lost infectivity could change the cells to be resistant to interferon. Treatment with anti-HVJ serum neutralized the ability of virus to induce the reversion. No host cellular protein synthesis associated with the HVJ superinfection was involved in this phenomenon. The effect of envelope fusion of paramyxoviruses on interferon action will be discussed.

R27.14

INTRALESIONAL THERAPY OF CONDYLOMATA ACUMINATA WITH NATURAL ALPHA-INTERFERON
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158 patients with genital warts were treated with either Alferon (affinity chromatography purified natural α-interferon) or placebo twice weekly for eight weeks or until warts completely disappeared, whichever came first. Interferon was injected into the bases of lesions at a dose of 2.5-5x105 IU/25mm2 wart area. The median total dose of interferon was 14.3x106 IU for a course of therapy; the median number of warts treated per patient was 4. Patients were evaluated for at least 3 and, in most cases, for 12 months following the completion of therapy.

Warts disappeared completely in 62% of interferon recipients and 21% of placebo recipients (p<.001). One-third of interferon recipients experienced a flu-like syndrome briefly after injections, disappearing within the first four weeks of therapy. For patients with recurrences after prior therapy, warts resolved completely in 73% of those who received interferon compared with none of those who received placebo (p<.001); in those whose warts were refractory to prior therapy, lesions resolved completely in 54% of the interferon group vs 22% of those who received placebo (p<.05). For those complete responders with 12 months' follow-up, recurrence rates were 25% in the interferon group vs 23% in the placebo group. Natural α-interferon, even in pretreated patients, was highly effective in eliminating genital warts.

R27.15

INTERFERON INDUCTION BY VSV IS NOT DUE TO A SINGLE MUTATION LEADING TO TEMPERATURE SENSITIVITY.
Margaret J. Sekellick and Philip I. Marcus
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Ts-mutants of VSV are either good inducers of interferon (IFN), or like their W+-parents, poor inducers. 5-FU mutagenized ts-mutants of Glasgow origin were very good inducers of IFN, but spontaneous ts-mutants of Orsay origin were poor or non-inducers. To determine whether chemical mutagenesis was responsible for this difference, we compared the IFN inducing capacity of spontaneous and 5-FU mutagenized Orsay and Glasgow ts-mutants. Of 12 spontaneous ts-mutants examined, regardless of origin, all were poor inducers. In contrast, 5-FU produced 1 of 5 Glasgow, and 2 of 8 Orsay-derived, ts-mutants that were good inducers of IFN. Thus, a single base change resulting in temperature sensitivity does not result in the acquisition of IFN inducibility. We hypothesize that several mutational events, or one rare one, are required to bring about the loss of IFN induction-suppressing activity intrinsic to all W+-VSV. Glasgow W+ was more sensitive to the lethal action of 5-FU, and may be more sensitive than Orsay W+ to the mutational event(s) required for IFN inducibility.

R27.16

INTERFERON SYSTEM: EFFECTS OF 2-AMINOPURINE.
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2-aminopurine, an indiscriminant inhibitor of kinases, results in a general reduction of phosphorylation in vivo, probably due to endogenous phosphatases. At low concentrations of 2-AP (1mM) interferon(IFN) induction in mouse L cells was stimulated up to 3-fold upon infection with VSV-T1026R1. At higher concentrations of 2-AP the yield of IFN was reduced in a dose-dependent manner from cells exposed to the drug during the 24 hr induction period. There was a 43-fold reduction in IFN yield at 15mM 2-AP. Primary chick embryo cells behaved similarly, but only after prolonged exposure to 2-AP. In both mouse and chick cells, 2-AP had no effect on cell viability, but in the latter case the action of IFN was compromised. These data implicate phosphorylation in IFN induction, possibly by blocking activation of cellular dsRNA binding proteins, like PKifn, which have been postulated to act as receptors in the cytosol during an early recognition step in the IFN induction process (Interferon 5, Ed. I.Gresser. Academic Press, p115-180, 1983).

R27.17

SYNTHESIS OF VESICULAR STOMATITIS VIRAL mRNAS IN VITRO BY VIRAL CORES ISOLATED FROM INTERFERON-TREATED CELLS.
G.C. Sen[1], D. Chattopadhyay[2], R. Kumar[1], A.K. Banerjee[2].
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Pretreatment of mouse JLSV-11 cells with 500 U/ml of interferon (IFN) reduces the yield of vesicular stomatitis virus (VSV) by about 5000 fold. Our earlier studies indicated impaired viral primary transcription to be the cause of inhibition of VSV replication in IFN-treated cells. In an attempt to reproduce in vitro the observed phenomenon of inhibition, we isolated viral cores from IFN-treated and untreated cells and compared their abilities to transcribe in vitro. Both cells were treated with anisomycin to restrict viral cores to primary transcription. The VSV cores isolated from IFN-treated cells (RNPI) synthesized about 10 fold less mRNAs in vitro as compared to cores isolated from control cells (RNPC). RNPI appears to contain a dominant inhibitor since when added to RNPC, it reduced the yield of VSV mRNA synthesized by the latter. This inhibitor appears to be an associated ribonuclease activity since VSV mRNAs presynthesized by RNPC or by virion cores were degraded upon incubation with RNPI but not with RNPC. Studies are in progress to characterize the RNPI-associated putative ribonuclease. (Supported in part by grants from the National Institutes of Health).

R27.18

REDUCED RECEPTOR BINDING BY HUMAN IFNg FRAGMENT LACKING 10 CARBOXY TERMINAL AMINO ACIDS.
P.O.Leinikki, J.Calderon and R.D. Schreiber. Institute of Biomedical Sciences, University of Tampere, Tampere, Finland and Washington University School of Medicine, St. Louis, USA.

Treatment of recombinant human interferon gamma (IFNg) with the arginine specific protease clostripain produces a stabile fragment with a molecular weight of 15.500. Structural analysis suggests that the cleavage occurs between residues 130 - 131 and thus produces a fragment that lacks 10 C-terminal amino acids. The fragmented and untreated molecules have identical N-terminal amino acid sequences and are equally reactive with either polyclonal or monoclonal anti-IFNg. Clostripain treated molecule displays more than 2000-fold reduction in its capacity to bind to cellular receptors at +4C. Functionally the fragment shows a 50-fold reduction in its ability to induce antiviral state or 10-fold reduction to induce Fc-receptors on U 937 cells.

These results suggest that the carboxy terminus contributes significantly to the affinity of the interaction between IFNg and its cellular receptors.

R27.19

INTERFERON INDUCERS INHIBIT GLUCOSE TRANSPORT SYSTEM OF EHRLICH ASCITES TUMOUR CELL. Y.M. Choy, K.P. Fung, C.Y. Lee.
Department of Biochemistry, The Chinese University of Hong Kong, Shatin, Hong Kong.

The effects of interferon inducers, polyribinosinic-polyribocytidylic acid (poly I.C), statolon and tilorone, on the growth of Ehrlich ascites tumour cells were studied. Among these three, tilorone is the least effective in suppressing the growth of tumour cells. Multiple administration of poly I.C, statolon and tilorone on days 2, 4 and 6 to mice after tumour implantation failed to induce a high serum interferon titer, probably due to the development of hyporeactivity.

Glucose transport in tumour cells were also studied. The same regime of drug administration reduced the value of maximal uptake (V_{max}) of the transport process by about 20%. This change is statistically significant. The apparent half-saturation constant (K_m) value was however unaffected. When the ability of the control and inducer-treated cells to bind cytochalasin B was examined, it was observed that the number of glucose-sensitive cytochalasin B binding sites (B_n) was significantly reduced with drug treatment even though the apparent dissociation constant (K_d) for the binding of cytochalasin B remained unchanged.

R27.20

EFFECT OF INTERFERON ON BOVINE DIARRHEA VIRUS REPLICATION IN MDBK CELLS. D.M. Justewicz, R. Magar, and J. Lecomte, Virology Research Center, Institut Armand-Frappier, Laval, Québec, Canada H7N 4Z3.

Although interferons (IFNs) can inhibit the replication of a number of viruses, and IFNs can be produced by bovine cell cultures in response to various inducers, little is known of their ability to inhibit bovine viral diarrhea virus (BVDV) replication. In vitro experiments were done, using MDBK cells to determine if BVDV is sensitive to the effects of IFN. These cells were selected because they are permissive for BVDV and are highly responsive to human IFN-α in other viral systems. Pretreatment of cells with moderate levels of natural human IFN-α or IFN-β (10 IU/ml) reduced the yield of infectious virions (NADL $TCID_{50}$/ml) by about 100-fold, and, at higher levels of IFN treatment (50 and 100 IU/ml), virus growth was inhibited by a further 30- to 150-fold by IFN-α and IFN-β, respectively. Pretreatment with higher levels of IFN-α (500 and 1000 UI/ml) resulted in a final inhibition of 5000-fold over the virus yield of $10^{8.5}$ $TCID_{50}$/ml in untreated cultures. Treatment of cells with IFN-α mixed with IFN-β did not result in synergistic antiviral activity. Immunofluorescence studies with BVDV-specific antiserum revealed that fewer than 10% of cells pretreated with 100 IU/ml IFN-α were positive for the expression of viral proteins at 12 and 24 h after infection, whereas greater than 50 and 100% of untreated cells showed a fluorescent signal, respectively. The results suggest that IFN-induced mechanisms of inhibition of BVDV replication in MDBK cells act early during the viral replicative cycle.

R27.21

EFFECTS OF DIFFERENT DOSING REGIMENS OF rHuIFNß ON ANTIVIRAL ACTIVITY IN SIMIAN VARICELLA VIRUS INFECTED MONKEYS. K. Soike, Delta Regional Primate Research Center, Covington, LA, U.S.A. and C. Gloff, Triton Biosciences, Alameda, CA, U.S.A.

Recombinant human beta interferon (rHuIFNß) administered by intravenous (i.v.) injection is effective in inhibiting simian varicella virus infection in African green monkeys. Blood levels of rHuIFNß after i.v. injection peak early and decline rapidly. Injection of rHuIFNß by subcutaneous (s.c.) or intramuscular (i.m.) routes results in lower but more sustained blood levels. Antiviral efficacy of beta interferon treatment in simian varicella infected monkeys was investigated using various dosing regimens with s.c. or i.m. injections. Antiviral activity was demonstrated in relation to these dosing regimens along with pharmacokinetic data reporting serum levels of rHuIFNß. Daily treatment for 10 days begun 24 hours after virus inoculation was most effective in modifying the course of the disease. Repeated injections of rHuIFNß resulted in higher serum levels of interferon indicating an accumulative effect.

R27.22

INTERFERON-GAMMA (IFN-γ), INTERLEUKIN-2 (IL-2) AND B CELL DIFFERENTIATION FACTOR (BCDF) PRODUCTION IN X-LINKED LYMPHOPROLIFERATIVE SYNDROME (XLP). N. Yasuda, P. Lai, D. Purtilo, Dept. Pathology/Microbiology, Univ. Nebraska Omaha, NE 68105.

XLP is a model for understanding protective mechanisms against Epstein-Barr virus (EBV). Post-EBV infection, fatal infectious mononucleosis, acquired hypogammaglobulinemia or B cell lymphoma emerge. T, B and natural killer cellular defects are observed in survivors of XLP. We studied 12 males with XLP, 12 relatives, and 9 normal male controls for production of IFN-γ, IL-2, and BCDF after challenging their T cells with phytohemagglutinin (PHA) and antigens on autologous EBV-transformed B-lymphoblastoid cell lines (B-LCL). T cell supernatants from all subjects enhanced IgM production while reducing proliferation of the BCDF sensitive B-LCL derived SKw 6.4 cells. Experiments to assess production of IgM by SKw 6.4 and blocking by anti-IFN-γ antibodies showed BCDF enhanced IgM production, while IFN-γ reduced SKw 6.4 proliferation. BCDF activity and IL-2 production were comparable in all groups ($p>0.05$). Supernatants from T cells activated by autologous B-LCL from patients failed to suppress proliferation of SKw 6.4 cells ($p<0.05$) and the T cells stimulated by autologous B-LCL produced less IFN-γ than controls ($p<0.001$). PHA-activated T cells from patients and controls produced comparable amounts of IFN-γ ($p>0.05$). These findings explain in part EBV-induced lymphoproliferation. Supported by PHS CA30196.

R27.23

INTERFERON-RESPONSIVE ELEMENTS IN THE 5'-FLANKING REGION OF THE HUMAN 2-5A SYNTHETASE GENE. M. Rutherford[1,2], G. Duckworth-Rysiecki[1], B.R.G. Williams[1,2]. Division of Infectious Diseases[1], Research Institute, Hospital for Sick Children and Department of Medical Genetics[2], University of Toronto, Toronto, Ontario, Canada.

Interferon-alpha (IFN) treatment of cells rapidly activates 2'-5'-oligoadenylate synthetase (2-5A synthetase) gene transcription (Hannigan and Williams, EMBO J. 5, 1607, 1986). Sequences upstream of the human 2-5A synthetase gene were cloned into the HindIII site of pSV0cat. The resulting plasmids were transfected into COS-1 and CV-1 cells and tested for their ability to produce correctly initiated hybrid 2-5A synthetase-cat mRNA and CAT protein activity in response to IFN treatment of the cells. A number of 5' sequences promoted 10-fold (in CV-1 cells) to 30-fold (in COS-1 cells) induction in CAT activity only in response to IFN treatment of the transfected cells. These sequences include a region of the 2-5A synthetase promoter which shows homology to (at 9 out of 13 positions) the core consensus sequence which occurs upstream of other IFN-inducible genes such as human metallothionein IIA. Constructions in which sequences immediately 3' of this region have been deleted fail to respond to IFN. The level of IFN-induced CAT activity produced by the 2-5A synthetase promoter (cloned into pSV0cat) was similar to that produced by the SV40 promoter (in pSV2cat) when assayed in COS-1 cells. CAT activities observed in pSV2cat transfected COS-1 and CV-1 cells were independent of IFN activity.

R27.24

ISOLATION OF CLONAL DERIVATIVES OF RD-114 CELLS WHICH RESPOND TO INTERFERONS DIFFERENTLY. R. Kumar, R. Tiwari, G.C. Sen. Molecular Biology Program, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, N. Y. 10021, U.S.A.

RD-114, a human rhabdomyosarcoma line, does not exhibit the full repertoire of responses to interferons (IFN). For example, in these cells replication of retroviruses but not of vesicular stomatitis virus (VSV) or of encephalomyocarditis virus (EMCV) is inhibited by IFN. Similarly induction of some of the IFN-inducible mRNAs but not of others is poor in these cells. Recently we have isolated several clonal derivatives of this cell line. In eight of these, we systematically examined the patterns of inhibition of replication of VSV, EMCV and retroviruses and of induction of 2-5(A) synthetase mRNA in response to IFN-α and IFN-γ. We observed that there was a great diversity in the spectra of responses among these clones. For example, EMCV replication was strongly inhibited in clone A2 but not in clone B6 whereas VSV replication was strongly inhibited in clone B1 but not in clone A3. Our analyses also revealed that induction of 2-5(A) synthetase mRNA cannot be directly correlated with inhibition of EMCV replication. The cell clones described here will be useful both for understanding the mechanism of IFN-mediated gene induction as well as for elucidating the IFN-mediated pathways involved in inhibition of replication of various viruses. (Supported by grants from the National Institutes of Health).

R27.25

PROTEIN ENGINEERING OF HUMAN INTERFERON GAMMA. W.C. Leung, G.Z. Jing & M.F.K. Leung. Depts. of Medicine, Medical Microbiology & Infectious Diseases, University of Alberta, Edmonton, Alberta T6G 2G3, Canada.

We previously chemically synthesized the structural gene for HuIFN-γ with modified nucleotide sequences to accomodate additional restriction endonuclease sites. Site specific mutants were generated by fragment replacement method. The gene was expressed in E.coli and served as a quick screen method. The mutants of interest were then ligated under an SV40 promoter and used to transfect the CHO cells. The intracellular as well as secreted form of expressed HuIFN-γ activity were assayed. Since the 3-dimensional structure for HuIFN-γ was not available, we based the mutagenesis strategy on a predicted secondary structure. The first objective is to localize regions on the protein molecule which affect its biological activities. We chose to generate mutants with minimal, but definite, conformational changes. Out of 30 mutant genes constructed, seven of them were able to re-express into biological active molecules. These mutants include deletion of N-terminal gln; deletion of amino acids 27-30, substitution of gly with glu at amino acid 31; and insertion mutants at 121 and 122 to extend alpha helix, etc. The antiviral activities of these mutants exhibit significant changes, ranging from 4 fold reduction to 10 fold enhancement. This study served to illustrate the feasibility of protein engineering on HuIFN-γ which will result in construction of mutants with significant changes on biological activity.

R27.26

A RELATIONSHIP BETWEEN FEVER AND INTERFERON GAMMA PRODUCTION. J.F. Downing[1], M.W. Taylor[2], R.S. Elizondo[2], K.M. Wei[2], and E. Walker[1]. Department of Biology[1], Physiology[2], Medicine[3], Indiana University, Bloomington, IN 47405, U.S.A.

Most viral infections are accompanied by fever, but very little is known of the effect of fever on the immune system. The effect of in vivo hyperthermia on plasma interferon levels, and interferon gamma induction was examined. Subjects were immersed in a warm water bath (40-45°C) to induce a steady rise in core (T_{re}) body temperature. Peripheral blood samples were obtained at T_{re} equal to 37° or 39°C. A significant increase (3-10x) was observed in IFN-γ production with increased body temperatures when PBML were stimulated with PHA or staphyloccocus enterotoxin B. There was little change in the plasma interferon levels.

Further studies indicated that in vivo hyperthermia was accompanied by other alterations in the immune system. Utilizing a fluorescent activated cell sorter, it could be shown that there was a change in T-cell subsets, and an increase in NK cells during hyperthermia. Utilizing a very sensitive radioimmune assay, the amount of interferon γ producing cells could be shown to increase from 0.75 -1.5%. However, this does not account for the 3-10 fold increase in IFN-γ production.

R27.27

RECOMBINANT ALPHA-2 INTERFERON GEL IN THE TREATMENT OF RECURRENT HERPES GENITALIS. S.L.Sacks [1], M. Conant [2], J. Connor [3], J. Douglas [4], L. Eron [5], M. Lebwohl [6], S. Marlowe [7], J. Mendelson [8], P. Bradstreet [9], D. Fedorczyk [10], J.R. Trout [ ], T. Plasse [9]. U. British Columbia, Vancouver, BC [1], Universities of California, San Francisco [2] and San Diego [3], CA, Denver Health and Hospital Corp., Denver, CO [4], Mt. Sinai School of Medicine, NY, NY [5], Fairfax, VA [6], Atlanta, GA [7], Jewish General Hospital, Montreal, PQ [8], Interferon Sciences, Inc.[9] and Rutgers University [10], New Brunswick NJ.

In order to evaluate the effects of topical recombinant alpha-2 interferon gel (5 million IU/g) in the treatment of recurrent genital herpes (GH), we conducted a randomized, multicentre, double-blind, patient-initiated, lesion-initiated trial in 125 men and 119 women. Both treatment groups were equally distributed in terms of sex, racial background, sexual preference, age, previous disease duration and severity, duration of last episode, and lesion size at entry. Each participant applied approximately 1 g of gel daily in 4 divided doses for 4 days. 143 alpha interferon (*a*) and 101 placebo(P) recipients with previously culture positive (CP) GH were examined and cultured daily for 5 days and at scheduled intervals thereafter to healing. The majority of patients were CP during the study episode [79% (*a*), 77% (P)]. Of tested viral isolates, 98% were type 2.

Among CP recipients (both sexes), the mean duration of pain was 4.1 days (*a*) and 5.7 days (P) ($P = 0.05$). The duration of itching in CP men was reduced in *a* recipients to 3.8 days compared with 6.1 days for P recipients. ($P = 0.05$). The duration of viral shedding (both sexes) from all lesions was reduced from a mean of 4.6 days (P) to 3.6 days (*a*) ($P = 0.02$) and also reduced from presenting lesions from 4.1 (P) to 3.3 days (*a*) ($P = 0.01$). Adverse effects were rare and occurred with equal frequency in both treatment groups.

We conclude that treatment with topical recombinant alpha-2 interferon gel was well-tolerated and significantly shortened the duration of itching in CP men, and reduced the durations of viral shedding and lesion pain in CP recipients.

R27.28

INHIBITION OF AFRICAN SWINE FEVER VIRUS BY INTERFERON: COMBINED INTERACTION OF INTERFERON WITH IDU AND (S)-HPMPA. Carmen Gil-Fernández and Eduardo Páez. Department of Virology. Centro de Investigaciones Biológicas. Velázquez 144. 28006 Madrid. SPAIN.

The antiviral effect of human α-interferon (IFN), alone or in combination with 5-iodo-2'-deoxyuridine (IDU) and (S)-9-(3-hydroxy-2-phosphonylmethoxypropyl)-adenine (S)-HPMPA has been studied in long-term cultures of African swine fever virus infected Vero cells. The animal inhibitory concentration of IFN, IDU and (S)-HPMPA are 50 units/ml, 1 μg/ml and 0.01 μg/ml. respectively. Reversibility experiments have shown that in the presence of IDU (50 μg/ml), viral reversion occurs and in some cases, a persistent infection is established. With (S)-HPMPA (5 μg/ml) there is no viral reversion but alterations of the cellular morphology are observed several days after drug treatment. However, when these drugs are used in combination with IFN (200 μg/ml), neither viral reversion nor alterations of cellular morphology occur.

These findings indicate a synergistic antiviral effect between IFN and IDU and an interesting IFN-mediated prevention of (S)-HPMPA-induced cellular alterations.

R27.29

VIRAL "PROSONE" IN THE MACROPHAGE CELL LINE P388D$_1$ IS MEDIATED BY INTERFERON.

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University of Peradeniya, Peradeniya, Sri-Lanka.

The macrophage cell line P388D$_1$ has been used to investigate antibody mediated enhancement of viral replication(1). Many arboviruses including West Nile(WNV) virus replicates in P388D$_1$ cells producing a cytopathic effect(CPE). It was observed that when P388D$_1$ cells were infected at high multiplicity of infection (MOI-1.0) with mouse brain or P388D$_1$ cell passaged virus (WNV or Ratai), CPE was inhibited via a viral "prosone". A comparable phenomenon was not seen in non macrophage cells of mouse (Loella) or simian(Vero) origin. The prosone inducing (PI) factor was pH 2 stable and the ultracentrifuged supernatant of WNV mouse brain virus retained PI activity indicating that defective interfering particles were not involved. Rabbit antibody to mouse / interferon(NIAID/Bethesda) abolished the prosone while control rabbit serum did not. Studies on virus yield in supernatants of P388D$_1$ cells infected prosone inducing viral inputs showed that though viral CPE was inhibited viral replication was not. We conclude that interferon present in or induced at high virus MOI results in an apparent virus "prosone" on the macrophage cell P388D$_1$.

Ref:1) Peiris & Portarfield (1979) Nature 282, 509

R27.30

EFFECTS OF TUMOR NECROSIS FACTOR ON HUMAN CYTOMEGALOVIRUS INFECTED FIBROBLASTS. J. Chehimi, M. Chun, D. Emanuel Memorial Sloan Kettering Cancer Center, New York, Usa

The effects of recombinant Human Tumor Necrosis Factor (rHu-TNF) on the ability of Human Cytomegalovirus (H-CMV) to infect human embryonic lung fibroblasts (MRC-5) were examined. Pretreatment of confluent MRC-5 monolayers with TNF 24 hours prior to infection with H-CMV (strain AD-169) inhibited viral plaque formation in a dose dependent manner up to 90%. No protection was seen when MRC-5 were pretreated for 2 hours.
Cells infected with H-CMV and treated with TNF had a significantly reduced viability 72 hours post infection, when compared to infected cells without TNF treatment (30%vs70%). These results suggest that TNF might induce an antiviral resistance in uninfected cells and TNF might selectively induce cytolysis of H-CMV infected fibroblasts.

R27.31

ANTIVIRAL EFFECTS OF 2',5' OLIGOADENYLATES (2-5As), ADENYL(3'-5')ADENOSINE (3-5A) AND ADENINE NUCLEOTIDES AGAINST RNA VIRUSES. A.Tominaga, S.Saito and S.Kohno. Department of Measles Virus, National Institute of Health, Tokyo, Japan.

The 2-5As are known to have important roles in the mechanism of interferon's antiviral action. In order to examine the antiviral effect of various nucleotides in nonpermeabilized cells, the 2-5As, 3-5A, adenine nucleotides (adenosine, ADP, ATP), and other nucleotides were added into the culture media of virus-infected FL cells. Three RNA viruses (VSV, sindbis and mengo viruses) were used as challenge viruses. Among the 2-5As, the dephosphorylated core of 2-5A dimer was most effective. At the concentration of 500 uM, it reduced the yield of each virus by greater than 99 %. However, phosphorylated 2-5A dimers had no effect against sindbis and mengo viruses, although they had a small effect against VSV. 3-5A and adenine nucleotides were also effective against sindbis virus and VSV, but these nuleotides were noneffective against mengo virus. The addition of 2-5A core, 3-5A and adenine nucleotides at early stage of viral replication was more effective than that at late stage. The other nucleotides, GTP, dTTP, UTP and CTP had no effect.

R27.32

THE INFLUENCE OF SMALL DOSAGES OF INTERFERON ON VIRUSES ON DIFFERENT STAGES OF CHRONIC HEPATITIS B.....Wu Zhang-qi, Xiong Kai-jun. Wuhan Institute of Virology, Academia Sinica and Tongji Medical University, Wuhan, PRC.

In this paper, 97 HBsAg and 60 HBeAg positive cases of chronic hepatitis B were treated with small dosages of interferon for one to six months. The viral targets were HBsAg, HBeAg, anti-HBe, DNAP and HBV-DNA. After one month of treatment, six targets had not improved conspicuously in 97 cases. After two months, DNAP and HBeAg positive rates declined conspicuously in only 77 cases, 5 viral targets all showed a statistically significant decline for HBV-DNA from 73.2% to 23.1% and HBsAg declined slowly from 100% to 50%. The viral targets were very conspicuous at this point. Out of six viral targets, DNAP and HBeAg were the most sensitive, the second sensitive was HBcAg and HBV-DNA was the third. However HBsAg was not highly sensitive. The results had shown that small dosages of interferon can control the reproduction of hepatitis viruses. A long course of treatment (over 5 months) is by all means more effective than a short course.

R28.1

IDENTIFICATION OF THE GENE FOR THE PRECURSOR OF ADENOVIRAL CORE PROTEIN X. J.M. Weber, C.W. Anderson. Department of Microbiology, Faculty of Medicine, University of Sherbrooke, Quebec, Canada and Biology Department, Brookhaven National Laboratory, Upton, N.Y. 11973, U.S.A.

Nucleoprotein complexes extracted from infected cells (Weber & Philipson, Virology 136, 321, 1984) or tsl virions grown at 39°C (Weber et al, Can. J. Micro. 29, 235, 1983) contain, in addition to core proteins V and pre-VII, an 11K polypeptide. To identify the gene coding for this protein, it was purified from tsl virions labeled with ^{35}S-met and ^{3}H-leu or ^{3}H-arg and subjected to N-terminal microsequencing. The sequence identified an open reading frame of 79 residues between nucleotides 17,679 and 17,915 in the L2 region which also codes for the other core proteins upstream of 79R, pre-VII and V. 79R is arginine rich (21%) and has a predicted molecular weight of 8715. It is cleaved by the viral endoprotease to virion protein X.

R28.2

The Electron Microscopy Examination of Adenovirus in stools of infants and children with diarrhoea

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Infectious diarrhoea in infant and children is most important part of human diarrhoeal diseases. It is caused by many bacteria, viruses and parasite. Among viral pathoges: rotavirus, fastidious(enteritic) adenovirus and small round virus are important. Many studies about diarrhoea in infant and children caused by rotavirus have been reported in China, but by adenovirus rarely. Our laboratory examined 337 fecal samples of infant and children with diarrhoea, which were collected from the pediatic clinic in Heilongjiang Province in 1982-1985. The 14 cases was observed adenovirus particles by EM and IEM. The positive rate is 3.69%. IEM was taken by serum of convalescent patient and normal infant, only convalescent serum was aggregated these adenovirus particles that was detected in stool. The result indicate that these adenovirus particles was related to diarrhoea of infant and children. We also found that some new viral fragments was aggregated with the typcal viral particles, when detected typical adenovirus particles in the fecals of infant and children with diarrhoea. The result indicated that it is common antigenic each other.

R28.3

ANTIBODY RESPONSE TO ENTERIC ADENOVIRUSES. T. Shinozaki, K. Araki, H. Ushijima, M. Kobayashi, R. Fujii, N. Okabe. Department of Pediatrics, Faculty of Medicine, Teikyo University, Infectious Diseases Division, Department of Pediatrics, National Children's Hospital, Tokyo, Japan.

Three hundred and twenty-two single and paired sera from children (newborn to 10 years old), young adults (18-20 years old), pregnant women and their cord sera, and elderly people (more than 70 years old) were tested for antibodies to enteric adenoviruses types 40 and 41 by neutralization test. Sera were also tested for a antibody to the common antigen of adenovirus by enzyme immunoassay.

The incidence of antibody rose gradually through childhood. Antibody was found in 20% of children between 1 and 6 months of age, and in 50% of those 37 to 48 months of age. Forty-eight percent of sera from young adults had antibody. Antibody was found in 10% of the aged. Nineteen percent of patients with acute gastroenteritis showed significant rise in antibody to Ad40 and/or 41. Forty-two percent of the same sera had significant rise to rotavirus by enzyme immunoassay.

R28.4

CO-TRANSFECTION OF TISSUE CULTURE BY EXTRACTS OF UROLOGICAL TUMOURS OR LYMPHOCYTES OF PATIENTS RESULTS IN COMPLEMENTATION OF TEMPERATURE-SENSITIVE ADENOVIRUS MUTANTS. J.Ongrádi, J.Farkas, P. Dán, G.Kulcsár, I.Nász, S.Csata. Institute of Microbiology, Semmelweis Medical University and St. Stephen Hospital, Budapest, Hungary

Both antibodies against early gene products of oncogenic adenoviruses and antigens in the tumour cells and in peripheral lymphocytes were found in 53 percent of patients with malignant urological tumours, while in 4 to 18 percent of controls. Infectious virus could be obtained from stimulated lymphocytes, occassionally. To verify partial adenovirus gene expression, in vitro complementation was carried out between extracts of tumour mass and two temperature-sensitive adenovirus mutants /tal8, tal9/ of type 5 using transfection of HEp-2 cells at restrictive temperature. 66-85 percent of extracts of renal adenocarcinoma, bladder carcinoma, bladder papilloma complemented one or both mutants, especially in case of women. Extracts of lymphocytes complemented in the same ratio. Seminoma, prostatic hypertrophy or non-tumourous extracts failed to complement. No recombination occured. Role for hormonal induction and mis-phosphorilation of gene products will be discussed.

R28.5

PHYSICAL ORGANIZATION OF THE ENTERIC ADENOVIRUS TYPE 41 EARLY REGION 1A. Annika Allard and Göran Wadell. Department of Virology, Umeå University, Umeå, Sweden.

Adenovirus type 40 (Ad40) and Ad41, subgenus F, are referred to as enteric adenoviruses (EAd's) since they are associated with infantile diarrhea. Like most other viruses causing diarrhea EAd's are fastidious with a limited capacity to grow in tissue cultures. However they can be propagated in selected tissue cultures, ie 293 cells that have been immortalized by transfection of Ad5 early regions E1A and E1B. The E1A gene products are termed immediate early gene functions and are synthesized constitutively in infected cells shortly after infection. The E1A products are involved in cellular transformation and are also required to positively regulate the transcription of the delayed early viral genes E1B, E2, E3 and E4.

The nucleotide sequence of Ad41 E1A has been determined. When compared to the corresponding region of wellcharacterized adenoviruses the size of the Ad41 E1A is more compressed with a remarkably short inverted terminal repeat (ITR) sequence. However the overall genetic organization is identical with three coding mRNAs resulting in a 51aa, a 220aa and a 248aa protein. The 220aa and the 248aa proteins differ in their primary sequence only by 28aa unique to the 248aa protein. In comparison with the E1A coding mRNAs of Ad12, Ad7 or Ad5 (subgenus A, B and C) there is a 50 % homology in the nucleotide sequence.

R28.6

Molecular Epidemiology and Genetic Variability of Adenovirus 3,4 and 7. Quan-gen Li[1] and Göran Wadell[2]. [1] Dept. of Virology, 302nd Hospital, Beijing, China. [2] Dept. of Virology, Umeå University, Umeå, Sweden.

A total of 18 different genome types of Ad3 and 15 genome of Ad7 were identified among 105 strains isolated from six continents by using 12 restriction endonucleases, (REs). The degree of genetic relatedness between the 33 Ad3 and Ad7 genome types was measured by pairwise comigrating restriction fragment (PCRF) analysis and projected in a 3D form. This method is particularly useful to acertain the genetic relatedness between moderate to closely related genomes. The intermediate strains Ad3-16 and Ad3-7 did not occupy intermediate positions but were members of the 1st and 3rd PCRF clusters of Ad3, respectively. In a worldwide survey of Ad3 and Ad7 strains the 3rd PCRF cluster of Ad3 occurred in Asia, North America and Australia, whereas the 1st PCRF cluster of Ad3 (Ad3p) appeared in Europe, South America and Africa. The 2nd PCRF cluster of Ad3 was only found in Africa. The 1st Ad7 PCRF cluster is rarely isolated; the 2nd Ad7 PCRF cluster is only found in China, the 3rd Ad7 PCRF cluster predominates among all pathogenic strains isolated from five continents. Among Ad4 strains seven genome types grouped into two PCRF clusters were identified The 1st Ad4 PCRF cluster occurred in North America and Europe; the 2nd predominated in Asia but appeared on other continents. The genetic distance between the two Ad4 PCRF clusters revealed a genetic space of a size that could be compared to the entire subgenus D-23 serotypes and 10 intermediate strains.

R28.7

FURTHER CHARACTERIZATION OF AN ADENOVIRUS TYPE 2 FIBRE-DEFECTIVE TEMPERATURE SENSITIVE MUTANT (H2 ts 125)

Marie-Laure CAILLET-BRADIN, Pierre LEMAY and Pierre BOULANGER
Unité de Virologie Moléculaire, INSERM U. 233, Lille, France.

In order to determine which of the two mutations Leu -- Phe (105) or Ala -- Val (434) present in the H2 ts 125 mutant (Boudin et al., EMBO J. (1983), 2, 1921-1927) was responsible for the thermosensibility and for the alteration of the migration of the fibre in SDS-gels, the following experiments were done. Marker rescue would indicate that the Ala - Val mutation is implied in the ts phenotype. The use of antipeptides sera directed towards various parts of the fibre molecule permitted to localize, on HCl-hydrolysis products of the fibre, the alteration of migration phenotype in a peptide containing the same mutation. Genetic analysis of the genome of five revertants of this mutant by restriction enzyme analyses or sequence determination are also presented.

R28.8

CHARACTERIZATION OF THE MAJOR STRUCTURAL PROTEINS OF HEMORRHAGIC ENTERITIS VIRUS. J.V. van den Hurk, Veterinary Infectious Disease Organization, University of Saskatchewan, 124 Veterinary Road, Saskatoon, Canada S7N 0W0.

Structural proteins of hemorrhagic enteritis virus (HEV), a turkey adenovirus, were analyzed by polyacrylamide gel electrophoresis, Western blotting, enzyme-linked immunosorbent assays, and electron microscopy. The hexon, penton, penton base, fiber, IIIa, and core proteins were identified, and a nomenclature has been introduced for these proteins in accordance with the convention used for human adenoviruses. The HEV soluble proteins, hexon, penton, penton base, and fiber were separated by polyacrylamide gel electrophoresis under native conditions, and analyzed by Western blotting using monospecific and monoclonal antibodies. The observed migration profile of the HEV soluble proteins was completely different from that of the human adenovirus type 2 proteins. Electron microscopic studies of purified HEV virus and purified penton proteins showed that HEV had one fiber protein attached to the penton base protein and not two fiber proteins as has been found for most avian adenoviruses. In addition, only one fiber polypeptide was present on SDS-polyacrylamide gels in purified penton preparations.

R28.9

THE ENTERIC ADENOVIRUS TYPE 40; GENE CLONING, GENE MAPPING, AND SEQUENCING OF THE TRANSFORMING GENE. M. Ishino[1], M. Demura[1,*], Y. Sawada[1], T. Yaegashi[1], T. Enoto[1], Y. Ohashi[1], S. Chiba[1], and K. Fujinaga[1], [1]Department of Molecular Biology, Cancer Research Institute, and [*]Department of Paediatrics, Sapporo medical college, Sapporo, Japan.

The Enteric Adenovirus type 40 (Ad40) is a fastidious virus, which barely propagates in conventional cultured human cells. Ad40 DNA was prepared from virions isolated from an outbreak of infantile gastroenteritis in Sapporo (Lancet 2, 954, 1983). BamHI fragments of Ad40 were cloned into plasmid vectors and physical maps of Ad40 genome with several restriction endonucleases were constructed, revealing that the Sapporo strain is a new variant. The BamHI-F located on the leftend 5.3% of the genome showed a weak and incomplete transforming ability on rat 3Y1 cell culture. The inverted terminal repeat of Ad40, 163 nucleotides long, relates most closely to that of Ad5. The E1A region has the genetic structure similar to that of other human adenoviruses such as Ad5 and Ad12, coding for at least two putative polypeptides of 27.5K and 24.3K. The trans-acting function of Ad40 E1A was also shown by cotransfection experiments for transient expression of chloramphenicol acetyltransferase gene linked with adenovirus E2 or E3 promoter segment.

R28.10

MAPPING OF ADENOVIRUS TYPE 2 FIBRE EPITOPES. G. Watson, P. Braddock, M. Burdon, W.C. Russell. Department of Biochemistry and Microbiology, University of St. Andrews, Fife, Scotland.

DNA's encoding various parts of the Ad2 fibre gene have been inserted into the β-galactosidase multiple cloning site of pUC9 and the resulting recombinant plasmids analysed for production of fibre antigens using both polyclonal serum and a bank of monoclonals made against the Ad2 fibre. It has been shown that one such recombinant encoding 200 amino acids at the C terminus of the fibre (which itself contains a total of 582 amino acids) can react both in RIA and blotting assays with the polyclonal serum and with five monoclonals all of which react in a type specific manner (i.e. with Ad2 and not with Ad5 fibre). This result is consistent with the model proposed by Green et al. (1983) which suggested that the knob portion of the fibre was encoded by the C terminal region of the gene. Subcloning experiments have shown that a recombinant encoding the 62 residue N terminal portion of this fragment no longer codes for the type specific epitope and further studies are underway to map more precisely both type and group specific epitopes.

Green, N.M., N.G. Wrigley, W.C. Russell, S.R. Martin and A.D. McLachlan. The EMBO Journal 2, 1357-1365, 1983.

R28.11

AN ANALYSIS OF Z-DNA BINDING PROTEINS IN ADENOVIRUS INFECTED CELLS. W.C. Russell, I. Leith, A. Beveridge, R.T. Hay. Department of Biochemistry and Microbiology, University of St. Andrews, Fife, Scotland.

A protocol for the ready detection of Z-DNA binding proteins was established using gel retention by Z-DNA antibody of a radiolabelled synthetic oligonucleotide in the Z configuration. This procedure was applied to cytoplasmic and nuclear extracts of both adenovirus infected and uninfected HeLa cells which had been fractionated using FPLC. Specific fractions with the ability to bind the labelled probe were detected and have been further characterised in terms of polypeptide content. The role of these binding proteins in both infected and uninfected cells is being studied.

R28.12

SEQUENCE OF THE ADENOVIRUS TYPE 40 HEXON AND THE SYNTHESIS OF OLIGONUCLEOTIDE PROBES USEFUL IN DIAGNOSIS. C. Toogood, R.T. Hay. Department of Biochemistry and Microbiology, University of St. Andrews, Fife, Scotland.

Our objective is to obtain nucleic acid probes which can be used to detect adenovirus types 40 and 41 in clinical material. We have thus identified unique sequences for use as probes to detect viral nucleic acids.

A DNA fragment from the N terminal region of the Ad2 hexon was used to probe Southern blots of restriction enzyme digested Ad40 and 41 DNA. In each case a single hybridising species was identified and cloned. These fragments were sequenced by the M13 dideoxy procedure. We now have the complete Ad40 hexon sequence and the N-terminal half of the Ad41 hexon. Oligonucleotides corresponding to unique regions of Ad40 and 41 were synthesised, labelled with ^{32}P and used to probe DNA from Ad40 and 41 and representatives from each adenovirus subgroup. Each group detected its respective DNA. The Ad40 probe did not cross-hybridise with any other adenovirus, while the Ad41 probe weakly recognised the Ad40 DNA.

Comparison of the Ad40 hexon amino acid sequence with that of Ad2 indicates that the hexons are likely to have a similar structure. The 3-D structure of the Ad2 hexon has recently been described (Roberts et al., Science (1986) 232, 1148-1151) and in collaboration with Dr. R. Burnett the Ad40 hexon sequence is being analysed by molecular modelling in the hope that a structure can be predicted.

R28.13

DESCRIPTION OF 8 NEW HUMAN ADENOVIRUS SEROTYPES OF SUBGENUS D (TYPES 42-49) FROM IMMUNOCOMPROMISED PATIENTS. John Hierholzer, Larry Anderson, Centers for Disease Control, Atlanta, GA, U.S.A.; Jonathan Gold, Sloan-Kettering Cancer Center, New York, NY; Thomas Adrian, Reinhard Wigand, Universitat des Saarlandes, Homburg/Saar, Fed. Rep. Germany.

Many adenoviruses recovered from immunocompromised patients, including renal transplant recipients, cancer patients, and AIDS patients, are unusual in their antigenic and genetic compositions. Isolates from urine are usually of Subgenus B and are variable in their serotype: 11, 11/H7+11, 16, 21/H21+35, 34, 34/H7+11, 35, 35/H11, and 35/H11+35. Isolates from stool are usually of Subgenus D and also are highly variable: 22, 26, 28, 29, 29/H17+28+29, 30, 30/Hx, 37/H17, 38, 39/H25+28 of the strains typeable by neutralization, hemagglutination-inhibition, and restriction enzyme analyses. The remaining Subgenus D isolates compromise 8 new serotypes and some additional intermediate strains. The new serotypes possess limited cross-reactions by reciprocal neutralization tests with existing types, mostly 10,20,25,26,27,39, and limited cross-reactions by reciprocal HI tests with types 10, 17, 20, 25, 28, 32, 38, 39. Restriction enzyme patterns with 7 enzymes showed additional relationships to existing prototypes but many unique patterns too.

R28.14

ANALYSIS OF NATURAL ANTIGENIC VARIATION IN ADENOVIRUS TYPE 2. Joseph P. Icenogle, John C. Hierholzer, Michael J. McDonough, Olen M. Kew. Centers for Disease Control, Atlanta, GA, USA.

We have documented antigenic variation in five adenovirus type 2 clinical isolates obtained from 1955 through 1985. These isolates have significantly reduced serum neutralization and hemagglutinin inhibition titers using antiserum to the prototype 1953 isolate (reductions are up to 4-fold and 8-fold, respectively). Nearly all amino acid sequence differences in the major capsid protein (hexon: 720 copies/virion) of adenovirus types 2 and 5 (both subgroup C) are encoded within a 1000-base pair region of the hexon gene (Kinlock et al., 1984), and map to the surface of the hexon trimer (Roberts et al., 1986). Thus, we sequenced the corresponding genomic region of the five adenovirus type 2 antigenic variants. Surprisingly, after accumulation of 3500 nucleotides of information, all amino acid and nucleotide sequences within this region were identical for the five variants. Therefore, naturally occurring antigenic variation in adenovirus type 2 does not primarily involve mutation of the hexon surface sequences. Current work to locate the determinants of natural antigenic variation in adenovirus type 2 will be described.

R28.15

STRUCTURE-FUNCTION RELATIONSHIPS OF THE ADENOVIRUS TYPE 5 SINGLE-STRANDED DNA-BINDING PROTEIN. C. A. M. Neale, G. R. Kitchingman. Department of Virology and Molecular Biology, St. Jude Children's Research Hospital, Memphis, Tennessee, USA.

The single-stranded DNA-binding protein (DBP) of adenovirus type 5 (Ad5) is a multi-functional protein that is involved in the control of viral macromolecular synthesis. To define the domains of the protein involved in each of the functions, we have isolated site-directed point and deletion mutants in the gene for the DBP.

Mutagenesis of aromatic amino acid residues in two of the three conserved regions (CR) previously identified by comparative sequence analysis resulted in a decreased competence for the mutant DBPs in DNA replication and a decreased affinity for ssDNA. Mutagenesis of a charged amino acid residue in CR2 also resulted in a functionally deficient DBP. These mutant proteins were shown to be stable in vivo, and to be modified to the same extent as the wild-type DBP. Other point mutants in the three conserved regions are presently being analyzed.

Deletion of the entire CR3 results in an unstable protein. Deletion of the amino-terminal coding part of the DBP results in truncated proteins which are not capable of supporting DNA replication in vivo. Surprisingly, these mutant DBPs did not bind appreciably to ssDNA-cellulose, even though they produced proteins containing the known DNA-binding regions. The implications of these results in terms of DBP folding and function will be discussed.

R28.16

HOW DO SEQUENCE-SPECIFIC PROMOTER METHYLATIONS LEAD TO GENE INACTIVATION ? Birgitt Knust, Bernd Weisshaar, Ulrich Müller, Arnd Hoeveler, Dagmar Knebel, Sabine Achten, Klaus-Dieter Langner, Doris Renz, and Walter Doerfler, Institute of Genetics, University of Cologne, Cologne, Germany.

In a series of investigations we have demonstrated that a number of viral promoters can be inactivated by sequence-specific methylations (for review, Doerfler et al. Develop. Molec. Virology, 8, 193-222, 1986). It remains a challenging problem to elicit the mechanism by which promoter methylation can cause gene inactivation. Presently the following problems are being studied: i) Transactivation of methylation inactivated promoters by E1 functions of adenoviruses. -ii) Reactivation of methylation inactivated promoters by viral enhancers. -iii) Reactivation of inactive promoters by 5-azacytidine and stability of demethylated state. -iv) Are hemimethylated promoter sequences functional ? -v) Binding of specific proteins to unmethylated and sequence-specifically methylated promoters. -vi) Fixation of unmethylated or methylated promoter-gene constructs in the genome of mammalian cells. Stability of methylation patterns, genetic activity of integrated constructs. Answers to some of the problems will be presented.

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R28.17

INTERACTION OF AN ADENOVIRUS PROTEIN WITH HISTOCOMPATIBILITY ANTIGENS. H.-G. Burgert, W. Jefferies, S. Kvist. Department of Immunology, Swiss Institute for Experimental Cancer Research, CH-1066 Epalinges, Switzerland and Ludwig Institute for Cancer Research, Stockholm branch, box 60202, S-104 01 Stockholm, Sweden.

The E3/19K protein of adenovirus type 2 binds to human histocompatibility (HLA) class I antigens, and inhibits intracellular processing and cell surface expression of HLA antigens. This lowered amount of cell surface histocompatibility antigens correlates with a reduction in susceptibility to lysis of the target cells by cytolytic T lymphocytes. Thus, the E3/19K protein indirectly reduces the cellular immune recognition. This might be the mechanism involved in persistent infections caused by adenoviruses.

The interaction between the two proteins is non-covalent and does not involve disulfide-bridges or carbohydrates. Hybrid histocompatibility genes were constructed and their products were expressed in cells together with the E3/19K protein. This has allowed us to identify the $\alpha 1$ and the $\alpha 2$ domains of the histocompatibility antigens as responsible for binding the viral protein. These domains are also crucial for T cell recognition. The implications for adenovirus evolution and their ability to cause persistent infections will be discussed.

R28.18

ADENOVIRUS EARLY REGION 1A MODULATION OF INTERFERON ANTIVIRAL ACTIVITY. Kevin P. Anderson and Elizabeth Fennie. Genentech Inc., 460 Point San Bruno Blvd., South San Francisco, CA, 94080, U.S.A.

Human adenovirus is a DNA virus which replicates as efficiently in human A549 cells pretreated with human interferon-α2 (IFN) as in untreated A549 cells. Vesicular stomatitis virus (VSV) on the other hand is a negative strand RNA virus which is very sensitive to the effects of IFN treatment in A549 cells. However, IFN mediated inhibition of VSV replication is not observed in cells coinfected with both adenovirus and VSV.

Several adenovirus mutants were tested for their ability to prevent IFN mediated inhibition of VSV replication in coinfection experiments. Mutants defective for expression of DNA synthesis and late gene expression abrogated IFN antiviral activity as well as wild type virus. Expression of adenovirus early regions 1B, 2A, 3, and 4 were likewise not required for manifestation of this activity. However, mutants defective for expression of early region 1A could not prevent IFN mediated inhibition of VSV replication in coinfected cells. Experiments using mutants with specific alterations within early region 1A suggest that expression of the 289 amino acid product of the early region 1A encoded 13S mRNA is essential for abrogation of IFN antiviral activity. Experiments are in progress to determine if other viral or cellular genes are required for abrogation of IFN activity and what effect adenovirus infection might have on expression of cellular genes induced by IFN treatment.

R28.19

GENETIC VARIABILITY OF SUBGENUS C ADENOVIRUSES
U. Wolf, Th. Adrian, R. Wigand.
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AV from subgenus C (1,2,5 und 6) are associated with respiratory diseases and are frequently found in humans. Many isolates of these types were collected over a period of thirty years. The DNA of these strains was isolated from infected HeLa cells and analysed by seven restriction enzymes. Fragment patterns differing from that of the prototype were called restriction variants (R variants), the combination of R variants in a given strain obtained with several endonucleases is namend genome type. To localize the restriction sites on the genome, we set up physical maps. Number of investigated strains (in parenthesis number of genome types): AV1 156 (38); AV2 205 (65); AV5 105 (34); AV6 24 (13). These findings reflect a pronounced genetic variability of strains belonging to subgenus C. While these differences mainly could be found between the genome types and the corresponding prototypes, the variations among some of the genome types were small;this points to a common ancestor of the genome types.

R28.20

GENOMIC POLYMORPHISM OF HUMAN ADENOVIRUSES
Th. Adrian, U. Wolf, R. Wigand.
Department of Virology, Faculty of Medicine, University of the Saarland, 6650 Homburg, FRG
The great number of AV serotypes (41),intermediate strains (e.g. 15/H9), and variants raises the question of the origin of new AV strains. We therefore analysed about 900 patient isolates, which were collected over a period of 30 years mainly in Europe and the USA. The viruses belonged to 4 subgenera. The DNA was analysed by 7 restriction endonucleases. Strains, which were indistinguishable in immunological tests, but showed different fragment patterns were named genome types. Types of subgenus C (AV1,2,5 and 6) were very variable, as also could be seen for AV3 and AV16 of subgenus B. Moderate differences showed genome types of subgenus B (AV7 and AV21),subgenus D (AV8 and AV37),and subgenus E (AV4). The variations were located on the genome and found to be distributed randomly. Our results lead to the following conclusions:
1. great differences were seen between the genome types and the corresponding prototypes.
2. only small differences were found among some of the genome types,which points to a common ancestor for them.

R28.21

INTERRELATIONSHIPS OF THE GENOMES OF HUMAN ADENOVIRUSES.
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Institute of Microbiology, Semmelweis University Medical School, Budapest, Hungary.
The genome of the prototype strain of human adenovirus type 35 (HAV 35) has been recently cloned and characterized by Kang, W.Gy., Berencsi, Gy.,Takács, M., Ascher, Z., Fejér, G., and Nász, I. (1987). The E1b region was shown to be more distantly related to representatives of other human adenovirus subgroups than E1b of HAV 7.
The association of HAV 35 with infections of immunocompromized patients suggested unique properties of E2a (coding for DNA-binding protein) and E3a/b (supposed to be involved in the inhibition of HLA expression; Pääbo, A., 1987) regions.
DNA homology could be detected of E2a sequences of HAV 35 to corresponding DNA fragments of representative types of subgroups B,C,D, and E of human adenoviruses. DNA fragments related to E3a/b of HAV 35 seem to be located at both termini of E3 within the genomes of subgroups C, D, and E. Detectable DNA homology of E3 was observed even to the corresponding regions of porcine AV 1, and bovine AV 2 genomes. Differences in E3 sequences of HAV 7 and 35 are still under investigation.

R28.22

A NEW VARIANT OF ADENOVIRUS TYPES 34 AND 35, ISOLATED FROM A KERATOCONJUNCTIVITIS PATIENT.
T.Yoshii, S.Yamazaki, K.Aoki, H.Sawada. NIH of Japan, Tokyo; Aoki Eye Clinic, Sapporo; Hokkaido Inst. Pub. Hlth, Sapporo, Japan.

An untypable adenovirus (AdV) was isolated from the eye swab of a patient with keratoconjunctivitis in Sapporo, Japan in 1984. The fresh isolate was not neutralized by type specific antisera against currently prevalent AdV types 1 through 8, 11, 19 and 37, but could be almost equally neutralized by the two reference antisera against AdV types 34 and 35, which were kindly supplied from Dr. Hierholzer. Furthermore, the freshly prepared rabbit antiserum against this isolate did almost equally neutralize all three strains; AdV 34, AdV 35, and the fresh isolate. However, agarose gel electrophoresis of viral DNA digested with restriction enzymes revealed that DNA patterns of the fresh isolate were distinguishable from those of AdV 34 and 35. The BglII-digested DNA pattern of the isolate was similer to that of AdV 34, but the Bam HI cleavage pattern was close to that of AdV 35. And finally, Kpn digestion could clearly distinguish the isolate from both AdV 34 and 35. These results indicate that this fresh isolate from an eye disease is a variant strain of either AdV 34 or AdV 35, or of both. The present finding may also provide evidence to suggest that AdV 34 and 35 as well as the present isolate should all be classified as the same type.

R29.1

REOVIRUS S OF *DROSOPHILA SIMULANS*, A HEREDITARY VIRUS RESPONSIBLE FOR A MORPHOLOGICAL CHARACTER.

López-Ferber, M., Louis, C., Vayrunes, J.C., Plus, N., Kuhl, G., Croizier, L., Comendador, M.A.*
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Genetical analysis of two distant *Drosophila simulans* natural populations revealed that the S morphological character (abnormalities of bristles) is maternally inherited and not sensitive to various antibiotics.(1).

Ultrastructural observations of S and normal flies showed reolike particles in subcuticular cells of the S category only.

Purification of the virions is difficult owing to the low yield characteristic of hereditary viruses. The electrophoresis of nucleic acid of prefixed virions revealed nine different bands; their pattern is different from those of the known reoviruses of Diptera.(2).

Our system seems a very interesting model for the study of virus-host relationships, particularly of the viral interactions on the development of the host sensorial organs.

(1) Comendador, M.A., Plus, N., Louis, C., López-Ferber, M. 1986. *Genet.Sel.Evol.* 18 (2) 131-144.
(2) Louis, C., López-Ferber, M., Comendador, M.A., Plus, N., in preparation.

R29.2

ANALYSES OF PROMOTERS OF THE AUTOGRAPHA CALIFORNICA NPV AND MAMESTRA BRASSICAE NPV POLYHEDRIN GENES. R. D. Possee and I.R. Cameron, N.E.R.C. Institute of Virology, Mansfield Road, Oxford OX1 3SR, United Kingdom.

The functioning of the Autographa californica nuclear polyhedrosis virus (AcNPV) polyhedrin gene promoter was analysed. Sequences upstream from the transcription start site were deleted using BAL31 exonuclease or additional short sequences added by inserting synthetic oligonucleotide linkers at restriction enzyme sites. The effect of these changes on polyhedrin promoter function was assessed by performing transient expression assays on the amount of polyhedrin mRNA produced in cells transfected with the plasmid and co-infected with a polyhedrin-defective helper virus. Alternatively, the polyhedrin gene in the plasmid was replaced with a ß-galactosidase (ß-gal) gene and the constructs co-transfected with wild-type AcNPV DNA to produce stable recombinants containing the modified promoter and ß-gal gene. Quantitative estimates of promoter activity were then made based on ß-gal activity and Northern blot analysis of mRNA.

The host specificity of polyhedrin promoters was also addressed by excising the putative promoter region from the Mamestra brassicae NPV genome and placing it before the ß-gal or polyhedrin genes in the AcNPV. Its ability to express each gene in infected cells was determined and compared with the activity seen in wild-type AcNPV-infected cells.

R29.3

ANALYSIS OF THE GENETIC ENVIRONMENT OF THE POLYHEDRIN GENE FROM THE NUCLEAR POLYHEDROSIS VIRUS OF MAMESTRA BRASSICAE. Iain R. Cameron, Rebecca Oakey, Benjamin Davies and Robert D. Possee. N.E.R.C. Institute of Virology, Mansfield Road, Oxford OX1 3SR, United Kingdom.

The genetic organisation of the Mamestra brassicae nuclear polyhedrosis virus (MbNPV) polyhedrin gene and its environs was investigated. The polyhedrin gene was cloned and sequenced. The DNA sequence was found to be 92% homologous to that of the closely related virus Panolis flammea NPV (PfNPV) and 82% homologous to the gene from the more distantly related Autographa californica NPV (AcNPV). Further sequencing showed that 111bp upstream of the polyhedrin gene open reading frame (ORF), and in the reverse orientation, is another ORF (ORF1) capable of coding a 183 amino acid protein. A homologous ORF1 was identified in the related PfNPV but not in AcNPV. The 5' end of the MbNPV polyhedrin mRNA was mapped by primer extension to a site 45bp upstream of the AUG codon. RNA isolated from Mamestra brassicae infected with MbNPV hybridises to a 950bp DNA fragment which spans ORF1 but does not overlap the polyhedrin mRNA. Transcripts from ORF1 and the polyhedrin genes of PfNPV and MbNPV were sized by Northern blotting. The 3' end of MbNPV polyhedrin mRNA maps near to a possible polyadenylation signal ATTAAA 220bp downstream of the coding sequence. These results are discussed with respect to differences in genetic organisation between baculoviruses.

R29.4

CODING AND INFECTION STRATEGY OF THOGOTO VIRUS. D. Staunton, C.R. Davies and P.A. Nuttall. N.E.R.C. Institute of Virology, Oxford, England.

Thogoto is a tick-borne virus originally isolated from a mixed population of Boophilus and Rhipicephalus ticks from cattle in Kenya. It has since been found througout Central Africa and Southern Europe. Thogoto virus is a candidate member of the Orthomyxoviridae; it buds from the plasma membrane and has a segmented single strand RNA genome.

A Sicilian isolate of Thogoto was purified for electron microscopy and RNA extraction. RNA segment profiles of the Sicilian and prototype isolates showed no discerible difference in segment mobilities.

cDNA recombinants, produced from RNA of the Sicilian isolate, were sequenced. The protein sequence was deduced and compared with available protein sequences of Influenza A, B and C.

Laboratory studies were undertaken to examine the interaction between Thogoto and a tick vector R.appendiculatus The virus pathway in infected ticks was studied using two techniques. Infected tick sections were labelled using immunofluorescence to determine the timecourse of viral antigen presence in different tick organs; and a cDNA recombinant was biotin labelled to probe tick sections for the presence of viral RNA.

R29.5

GENOMIC CHARACTERIZATION OF A BACULOVIRUS, A BIOLOGICAL CONTROL AGENT FOR THE INSECT PEST **ORYCTES RHINOCEROS(L).**.

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Indian Institute of Science, Bangalore 560012 (INDIA)

Oryctes rhinoceros (L) is a major insect pest of coconut and oil palms, distributed ubiquitously in the coconut growing tracts of the world. A Baculovirus infective to all the larval stages (three instars) and adult beetles has been isolated from Kerala (West coast of India) and succesfully used in field trials as a biological control agent. Ultra thin sections of midgut of infected beetles revealed enveloped bacilliform virions measuring 213-260 x 77-108 nm. The DNA isolated from the virus particles is covalently closed, circular and supercoiled. The DNA is 135 Kbp in size and contains 43 mole percent GC. The methylation pattern of the genomic DNA has been analysed using isoschizomeric restriction enzymes and by direct estimation of the methylated bases. The number of restriction sites and fragment size distribution with respect to the enzymes Eco RI, Bam HI, Hin dIII, Pst I, Cla I, Sal I, Xba I, Kpn I and Sma I have been determined. The Pst I fragments of the genome have been cloned and a physical map of the genome has been established using the above enzymes.

R29.6

TRANSCRIPTION FROM A NEWLY IDENTIFIED OPEN READING FRAME UPSTREAM FROM THE AUTOGRAPHA CALIFORNICA NPV POLYHEDRIN GENE. K. Gearing and R.D. Possee. NERC Institute of Virology, Mansfield Road, Oxford OX1 3SR United Kingdom.

The coding capacity of the Autographa californica nuclear polyhedrosis virus (AcNPV) genome immediately upstream from the polyhedrin gene has been examined. Nucleotide sequencing identified a 600bp open reading frame in the opposite orientation to the polyhedrin gene. This gene has an ATG translation start codon just 156bp from the ATG of the polyhedrin, which probably leads to an overlap of respective promoter functions. We investigated the expression of this gene using Northern blotting of mRNA from AcNPV-infected cells with strand specific probes. The 5' and 3' ends of the mRNA were mapped using S1 nuclease analysis and a further precise definition of the transcription start site made by primer extension on the mRNA. The susceptibility of the gene to metabolic inhibitors was also addressed in order to categorise its temporal control. Hybrid selection techniques were used to isolate mRNA for in vitro translation to identify the polypeptide appropriate to this gene. The data are discussed with special reference to the transcriptional control of this newly identified gene compared to that of the polyhedrin gene and whether there is any interdependence between them.

R29.7

A MOLECULAR BIOLOGICAL INVESTIGATION OF AN APHID PICORNAVIRUS. C. Williamson, M.B. von Wechmar and E.P. Rybicki.
Department of Microbiology, University of Cape Town, Rondebosch 7700, Cape Town, South Africa.

Rhopalosiphum padi virus (RhPV) is an aphid pathogen, detected in many aphid species. RhPV has a single-stranded, non-segmented RNA genome. Due to its physicochemical properties it has tentatively been classified as a Picornavirus. Further molecular characterisation of RhPV was performed in order to provide more data for classification. Additional virion proteins to those previously reported were identified by ^{125}I-labelling. Differential surface labelling of the virion gave information on the relative position of the capsid proteins. It appears that RhPV RNA has a 3' poly(A) tail. A characteristic of Picornaviruses is that their entire genome is translated into a single polypeptide. Translation of RhPV RNA was investigated using a rabbit reticulocyte lysate system and by immunoprecipitation of the products. Replicative form dsRNA was isolated from aphids and several dsRNA species were identified by electrophoresis. Fractionated dsRNA was blotted and probed with ^{32}P 5'-end labelled genomic RNA. Hybridisation confirmed that the dsRNA was of viral origin.

R29.8

THE USE OF SERUM REPLACEMENTS IN MEDIUM FOR THE IN VITRO PRODUCTION OF AN INSECT NPV. J. L. Vaughn, J. R. Adams, E. M. Dougherty and J. T. McClintock. Insect Pathology Laboratory, ARS, USDA, Beltsville, Md. 20705 USA

Fetal bovine serum, routinely used in medium for the production of nuclear polyhedrosis viruses, NPV, is highly variable, expensive, and frequently in short supply. Two commercially available controlled process serum replacements types 1 and 3 (CPRS-1 and CPRS-3, Sigma Chemical Company) were tested for use in the in vitro production of the Autographa californica NPV. The cell line used was the Spodoptera frugiperda line, IPLB-Sf-21AE. The medium was supplemented with 10% volume/volume of each replacement. Virus replication was evaluated by synthesis of viral peptides, release of nonoccluded virus and number of polyhedra produced. The quality of the polyhedra was evaluated by insect assay and electron microscope examination. Virus replication in CPRS-3 appeared normal. Large numbers of virions were produced, incorporation of virions into polyhedra appeared normal, and the number of polyhedra produced was equal to FBS. CPRS-1 was not equivalent to fetal bovine serum for support of virus replication. Although polyhedra production appeared high, the incorporation of virions was low. Many of the polyhedra contained no virions. Thus CPRS-3 appears to be an adequate replacement for FBS and provides the advantages of better uniformity and a more stable supply.

R29.9

COSMID CLONING AND RESTRICTION ENDONUCLEASE MAPPING OF THE DNA OF THE NUCLEAR POLYHEDROSIS VIRUS OF LYMANTRIA DISPAR, THE GYPSY MOTH. J. T. McClintock, and E. M. Dougherty. Insect Pathology Lab., ARS, USDA, Beltsville, MD 20705 USA

In order to study the organization and expression of the genome of the multiple-embedded nuclear polyhedrosis virus (NPV) of Lymantria dispar (LdMNPV), the gypsy moth, and as a prelude to transcriptional and translational maps, the DNA from a plaque-purified isolate of LdMNPV was partially characterized by restriction endonuclease analysis and a physical map constructed using cosmid cloning and in vitro lambdaphage packaging. Digestion of the viral DNA with BamHI, BglII, EcoRI, and HindIII resulted in distinguishable fragmentation profiles. Based on these results, the molecular weight of LdMNPV DNA was determined to be approximately 85.2 X 10^6 daltons or 129.1 kb. When LdMNPV DNA was digested with methylation-sensitive isoschizomers, the viral DNA did not appear to be extensively methylated, at least not at the sequences recognized by these restriction enzymes. The genome of LdMNPV was mapped by examining overlapping BamHI or PstI fragments from cosmid clones constructed from partial digests of viral DNA and by Southern cross hybridization. Using the EcoRI-I fragment of Autographa californica NPV to probe Southern blots of recombinant cosmid clones and restricted viral DNA, the polyhedrin gene was mapped to the LdMNPV genome.

R29.10

REGULATION OF THE AcMNPV OCCLUSION BODY GENE EXPRESSION. R.R. Cettig, B.L. Florence, P.H. Flore and H.A. Wood. Boyce Thompson Institute for Plant Research, Ithaca, New York, U.S.A.

The occlusion body (OB) gene of AcMNPV is one of the most characterized baculovirus genes. It is under the control of very strong promoter sequences. Transcription is temporally controlled and occurs late in the infection cycle.

Site directed mutagenesis of the OB gene has been used to construct OB minus mutants which lack translational start sites or produce truncated gene products. Several mutants which produce truncated gene products have been shown to totally inhibit the expression of the wild type OB gene during co-infection of tissue culture cells. However, during co-infection with the wild type virus, the truncated OB gene product of the mutant is expressed at high levels and is under normal temporal control. Data will be presented concerning the DNA sequence analysis of interfering and non-interfering OB genes and their control over wild type OB gene expression.

R29.11

A MODEL FOR ESTIMATING VIRUS REPLICATION RATES FROM LARVAL ASSAYS AND VALIDATION BY DNA:DNA HYBRIDIZATION DATA. N.A.M. Van Beek, P.H. Flore, H.A. Wood and P.R. Hughes, Boyce Thompson Institute at Cornell, Ithaca, NY, USA.

Successful use of baculoviruses in biological control programs depends on a detailed knowledge of the interaction of the virus and the target insect. After establishment of an infection the survival time depends on the effective dose employed, the in vivo rate of increase (α) and the concentration of virus at which response of the host is triggered (C). These parameters can be calculated from a simple but accurate bioassay by plotting median survival times against ln of dose, which gives an inverted S-shaped curve. The inverse of the slope of the linear part of this curve represents α; C is estimated from the point at which this line intersects the abscissa when dose is expressed as effective nucleocapsids. Division of ln 2 by α gives an estimate of doubling time. To provide an independent test of this model, DNA of Trichoplusia ni larvae was extracted at various time intervals after infection with AcMNPV and the amount of viral DNA was determined by DNA:DNA hybridization. Viral DNA increased exponentially at a rate of 0.264 h^{-1} (95% C.L. 0.246 and 0.282) to a maximum of 5 x 10^9 genomic copies at 48 h, which agrees well with the bioassay results of 0.314 h^{-1} (95% C.L. 0.263 and 0.387) and 7 x 10^9 for α and C, respectively.

R29.12

DEFECTIVE RNAs OF BLACK BEETLE VIRUS. Ranjit Dasgupta, Bernard Selling and Paul Kaesberg. Biophysics Laboratory, University of Wisconsin, Madison, Wisconsin 53706, USA.

The genome of Black beetle virus (BBV), a member of the Nodaviridae family, consists of two messenger active RNA molecules. BBV RNA1 encodes protein A, involved in viral RNA synthesis and protein B, whose function is unknown. RNA2 encodes the capsid protein. Although BBV is highly lytic to cultured Drosophila cells, about 1% of cells survive and become persistently infected. Virions purified from such cells contain defective RNAs in addition to RNAs 1 and 2. BBV defective RNAs therefore contain signals for replication and packaging. We have cloned and analyzed the sequence of six defective RNAs of BBV (W17 strain). Five of these RNAs originate from RNA 2 (1400 b) and are approximately half its size (650-700 b). The 5' half consists of the 5' RNA2 sequence with several large deletions and the 3' region consists of short, alternating RNA2 5' and 3' sequences. Virions derived from one persistently infected cell line contain a defective RNA which originates from RNA1 (3100 b) and is of RNA2 size. At least 200 bases from both 5' and 3' ends of RNA1 are conserved in this defective RNA.

R29.13

IN VIVO INFECTION OF LYMANTRIA DISPAR LARVAE WITH A NUCLEAR POLYHEDROSIS VIRUS OF AUTOGRAPHA CALIFORNICA (AcNPV). E. M. Dougherty, J. T. McClintock, K. S. Shields*. Insect Pathology Lab., ARS, USDA, Beltsville, Md., and *FS, USDA, Hamden, Conn., USA.

The infectivity of AcNPV was determined in L. dispar larvae by examining different routes of inoculation and virus treatments. First instar L. dispar larvae, surface fed a plaque-purified variant of AcNPV at an LC_{50} dosage, required 10^6 more polyhedral inclusion bodies (PIBs) than the homologous LdNPV, while second instar larvae were unaffected at any dose tested. In vitro dissolution of AcNPV did not enhance infectivity when L. dispar larvae were orally injected or surface fed liberated virus. However, intrahemocoelic inoculation was at least 100-fold more efficacious. Complementation studies with UV and heat-inactivated LdNPV did not enhance AcNPV activity. Intrahemocoelic injection of nonoccluded (NOV) AcNPV was highly efficacious producing mortality at dosages as low as 10 PFU/larva. Electron microscopic examination and hybridization studies of AcNPV-infected L. dispar larvae revealed virus replication in hemocytes, fat body, tracheal tissue and the hypodermis, however, no virus replication was detected in the midgut. Virus progeny were further characterized by quantifying NOV production by the endpoint dilution assay, PIB count, PIB bioassay against Trichoplusia ni and L. dispar larvae, electron microscopy, and restriction endonuclease analysis of progeny genomes.

R29.14

ISOLATION OF THE GENE CODING FOR THE MAJOR STRUCTURAL PROTEIN OF TIPULA IRIDESCENT VIRUS. S. Tajbakhsh[1,2], P.E. Leel, V.L. Seligy[2]. [1]Dept. of Biology, Carleton University; [2]Division of Biological Sciences, National Research Council, Ottawa, CANADA.

Tipula iridescent virus (TIV), a dsDNA virus, replicates cytoplasmically in insect cells and belongs to the Iridoviridae family. To better understand gene organization and expression in TIV, we chose to isolate the gene coding for the major TIV structural protein (~50kDa) and follow its expression in Estigmene acrea cells by indirect immunofluorescence and western blots using a polyclonal antibody.

To isolate this gene, we identified the most abundant TIV mRNA late in infection. Total RNA was extracted (4,8,12,16 and 24h p.i.) from infected E. acrea and Aedes albopictus cells and radiolabelled cDNA was made from this RNA. These probes were hybridized to replica blots of digested TIV DNA. Our analysis using both cell lines revealed that TIV DNA SalI fragments S24a (4.4kbp) and S36 (1.1kbp) were the most intensely hybridizing. Further, while the intensity of S24a was higher than S36, the intensity of S36 decreased in the middle of infection. A 2.3kbp clone of S24a in M13 mp19 which identifies a single RNA band on northerns is being characterized by expression and sequencing.

R29.15

A CONSTRUCTION OF A POSITIVE SELECTION EXPRESSION VECTOR FOR AUTOGRAPHA CALIFORNICA NUCLEAR POLYHEDROSIS VIRUS (AcNPV). John Kuzio and Peter Faulkner. Department of Microbiology and Immunology, Queen's University, Kingston, Ontario, Canada.

In order to simplify selection of recombinant genes in AcNPV, a positive selection vector using the alpha-complementation phenomenon of the E. coli enzyme beta-galactosidase (gal) was developed. Portions of the genome encoding the AcNPV genes for polyhedrin and p10 were modified to express z' and 'z components of gal.

A fragment of the gal gene containing the C-terminal 'z portion was inserted downstream of the initiation codon of the AcNPV p10 gene. The z' N-terminal portion of the gene was inserted upstream of the initiation codon of the AcNPV polyhedrin gene. When expressed, the z' polypeptide complements the 'z portion to produce an active beta-galactosidase enzyme. This vector, AcNPVz1, provides the basis for the positive selection system to be described.

R29.16

THE NUCLEAR MATRIX OF NORMAL AND BACULOVIRUS-INFECTED INSECT CELLS. Marijo E. Wilson, Department of Entomology, University of Kentucky, Lexington, KY, USA.

The nuclear matrix has been implicated in many cellular processes. In order to examine the role of the nuclear matrix in baculovirus replication and/or assembly, the purification protocols were developed and the polypeptide components of uninfected Spodoptera frugiperda cells and cells infected with the baculovirus Autographa californica nuclear polyhedrosis virus characterized. The nuclear matrix of uninfected cells contains six major polypeptides (134K, 125K, 75K, 59K, 52K and 30K) as well as several minor species. Nuclear matrices of infected cells are predominately composed of low molecular weight proteins (30K, 17K, 16K, and 14K). The 30K protein co-migrated with the occlusion-specific protein, polyhedrin, and was a major component of the nuclear matrix by 24 hours post-infection. Using a polyhedrin-deletion mutant the 30K protein has been conclusively demonstrated to be polyhedrin. The association of polyhedrin with the nuclear matrix in reference to viral packaging and the occlusion process will be discussed.

R29.17

FUNCTIONAL ANALYSIS OF THE POLYHEDRIN GENE PROMOTER OF BOMBYX MORI NUCLEAR POLYHEDROSIS VIRUS (BmNPV). R.G. Meidinger and K. Iatrou, Department of Medical Biochemistry, The University of Calgary, Calgary, Alberta, Canada

We have initiated the functional characterization of BmNPV polyhedrin gene promoter by deletion mutagenesis. Following Bal 31 digestion of a cloned polyhedrin gene, we have aquired a library of clones containing polyhedrin genes whose sequences around the 5'-terminus have been deleted to various degrees. Using a combination of restriction and hybridization assays, we classified the mutagenized polyhedrin genes according to the extent of the effected deletions. To assess the effect of the various deletions on the function of the polyhedrin promoter, we cloned a CAT gene into the deletion junctions of several of the mutant plasmids and transfected the resultant polyhedrin/CAT gene constructs into B. mori tissue culture cells which had been previously infected with wild type BmNPV. CAT assays employing extracts derived from the transfected cells revealed that, while deletion of seven nucleotides of the 5'-untranslated region of the polyhedrin gene has no effect on the activity of the polyhedrin promoter, deletion of a sequence encompassing the entire 5'-untranslated region and two nucleotides of the 5'-upstream sequences abolishes completely promoter function. We conclude that the sequences around the cap site of the gene represent essential components of the polyhedrin promoter.

Supported by the MRC of Canada and the Alberta Cancer Board.

R29.18

ISOLATION AND CHARACTERIZATION OF EARLY MUTANTS OF AcMNPV. S. W. Partington and E. B. Carstens. Department of Microbiology and Immunology, Queen's University, Kingston, Ontario, Canada.

The Baculovirus AcMNPV, mutagenized by 5-BudR, was screened for ts mutations affecting early gene functions. Four ts mutants (ts317, ts538, ts1203 and ts1293) were isolated. All failed to synthesize viral polyhedral inclusion bodies at 33°. Titration of supernatants from cells infected at 25° and 33° revealed the absence of infectious virion production at 33°. Cell spot hybridizations and restriction analysis of total intracellular DNA indicated viral DNA was synthesized by these ts mutants at 33°. Analysis of protein synthesis at 33° revealed that, while all four mutants turned off host cell protein synthesis, late viral protein production was aberrant. Late protein synthesis was transient in ts317, ts538 and ts1203, and greatly reduced in ts1293. Complementation of ts317 and ts538 with other known ts mutants has placed these mutants in separate and unique complementation groups. Marker rescue with cloned wildtype Eco RI fragments has tentatively mapped the ts lesion of ts317 and ts538 respectively within the 20.2-25.3 and the 42.4-51.7 map unit regions. Supported by N.R.C.

R29.19

ISOLATION AND CHARACTERIZATION OF SMALL RNA VIRUSES FROM FIELD COLLECTIONS OF EASTERN SPRUCE BUDWORM. T. Roberts, W. Kaupp, and P. Faulkner. Department of Microbiology and Immunology, Queen's University, Kingston, Ontario, and Forest Pest Management Institute, Sault Ste. Marie, Ontario, Canada.

Field collections of apparently healthy eastern spruce budworm (Choristoneura fumiferana) larvae, collected in Ontario, were surveyed for the presence of small RNA viruses. Infectious virus was isolated from pooled larval extracts by blind serial passage in cultured Drosophila melanogaster and C. fumiferana cell lines, from 10 to 21 collections tested. The vertebrate and invertebrate host range in vitro of these virus isolates has been determined. LD50's for 4th instar budworm was about 5×10^5 PFU/larva. Biochemical and serological analyses demonstrated close similarity between these isolates and the prototype insect picornavirus, cricket paralysis virus. Each virus isolate has a 37S poly-adenylated single-stranded RNA genome and three virion-specific structural proteins which co-migrate with cricket paralysis virus structural proteins. The in vitro infectivity of C. fumiferana virus isolates was inhibited by polyclonal anti-cricket paralysis virus sera. A comparative study of genomic sequence homologies between these virus isolates and cricket paralysis virus has been undertaken. This report further demonstrates the ubiquitous distribution among species, and the broad insect host range of the insect picornaviruses.

R29.21

PRODUCTION AND CHARACTERIZATION OF MONOCLONAL ANTIBODIES AGAINST STRUCTURAL PROTEINS OF THE OCCLUDED AND NONOCCLUDED PHENOTYPES OF AUTOGRAPHA CALIFORNICA NULTIPLY EMBEDDED NUCLEAR POLYHEDROSIS VIRUS (AcMNPV). M. Whitford and P. Faulkner. Department of Microbiology and Immunology, Queen's University, Kingston, Ontario, Canada.

A panel of monoclonal antibodies were produced against structural proteins of the nonoccluded (NOV) and occluded (OV) virus phenotypes of AcMNPV.

Of the forty-five monoclonal antibodies produced against NOV polypeptides, three reduced the infectivity of NOV in a neutralization assay and bound to a 67K polypeptide in an immunoblot. Seven hybridoma clones produced antibodies that reacted against OV in an indirect ELISA. Similarly, twenty-five monoclonal antibodies were made against structural polypeptides of the OV. Five of these antibodies recognized NOV in an indirect ELISA. The polypeptide specificity of 29 monoclonal antibodies produced against NOV and 24 monoclonal antibodies produced against OV were assigned using immunoblotting and radioimmunoprecipitation techniques. Preliminary studies were performed to identify monoclonal antibodies that recognized OV polypeptides.

R29.23

MOLECULAR CLONING OF SalI AND BamHI FRAGMENTS OF ATTACUS RICINI NUCLEAR POLYHEDROSIS VIRUS DNA AND LOCATION OF 10 YELLOW GENE ON THE ArNPV GENOME

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Department of Virology, Wuhan University, P.R.C.

In this paper, we reported that Attacus ricini nuclear polyhedrosis virus (ArNPV) DNA was completely digested by SalI, and EcoRI endonucleases into fragments of different sizes. These fragments as detected by electrophoresis on agarose gel to determine the molecular weight. Selected SalI and BamHI restriction fragments of ArNPV-DNA to be cloned into plasmid PBR322 and Ybr325 respectively. By using pMV-VI (a cDNA of polyhedrin gene mRNA of Autographa Californica nuclear polyhedrosis virus was a gift from prof. Lois K. Miller) as a probe, and were labeled with ^{32}P-dATP by nick translation. The Southern blot and hybridisation results demonstrated that the nucleotide sequence of SalI-II, HindIII-III and EcoRI-VI restriction fragments of ArNPV-DNA were homologous with the cDNA of mRNA of polyhedrin gene of AcNPV. And we suggested that these fragments might contain the coding sequence of the polyhedrin gene of the genome of ArNPV.

R29.26

OVERLAPPING SETS OF VIRAL RNAS REFLECT ARRAY OF POLYPEPTIDES IN THE ECORI-J AND-N FRAGMENTS (MAP POSITIONS 81.2 TO 85.0) OF THE AUTOGRAPHA CALIFORNICA NUCLEAR POLYHEDROSIS VIRUS GENOME. Cornelia Oellig, Brigitte Happ, Thomas Müller, and Walter Doerfler, Institute of Genetics, University of Cologne, Cologne, Germany.

In several parts of the Autographa californica nuclear polyhedrosis virus (AcNPV) genome, nested sets of overlapping RNAs with common 3' or 5' termini have been recognized. The pattern of viral transcription and the arrangement of viral gene products in the region of 81.2 to 85.0 map units were investigated. In this segment of the AcNPV genome, seven size classes of viral RNA with common 3' termini have been identified which range in size from 1.3 to 4.7 kb. The nucleotide sequence of this part of the AcNPV genome was determined. Computer analyses revealed several open reading frames (ORFs) on the rightward transcribed strand. The presumptive polypeptides encoded by the larger ORFs ranged in size from 11.3 to 33.6 kiloDaltons (kD). The amino acid sequence of the 33.6 kD polypeptide encoded by ORF3 showed a clustered sixteen fold repeat of the dipeptide arginine-serine. The results of in vitro translation experiments using hybrid-selected RNAs yielded polypeptides of approximately 28, 33, (38), and 50 kD. The localizations of individual size classes of RNAs in the 81.2 to 85.0 map unit region of the viral genome were determined precisely at the 3' and 5' termini by S1 protection analyses. (Aided by DFG through SFB74-C1).

R29.22

IMMUNODETECTION OF EUXOA SCANDENS RILEY CYTOPLASMIC POLYHEDROSIS VIRAL ANTIGENS IN INSECT TISSUES AND CELL LINES. G. Charpentier[1], R. Béliard[1], S. Belloncik[2], M. Arella[2], H. Stykowsky[3], S. Garzon[3]. (1) Université du Québec à Trois-Rivières, Trois-Rivières; (2) Centre de recherche en virologie, Institut Armand-Frappier, Laval-des-Rapides; (3) Université de Montréal, Montréal, Québec, Canada.

Using IgG from sera against 1° gut of infected larvae, 2° purified inclusion bodies, 3° dissolved inclusion bodies and 4° purified virus particles, we tried to detect viral antigens of cytoplasmic polyhedrosis virus (C.P.V.) of Euxoa scandens Riley (Lepidoptera: Noctuidae) in infected cells lines of E. scandens and Lymantria dispar. The various viral antigens were detected in both cells lines by the immunoperoxidase under the light microscope. The IF technique described kinetic of their appearances in both cells lines. The viral antigens were detected more rapidly in L. dispar cells than in E. scandens cells. We also used this technique to study the effect of temperature of incubation, viral inoculum concentration, cordycepin, and dual infection of a cell line with C.P.V. and Chilo iridescent virus on viral antigens appearance. We also used the immunocolloidal gold technique at an ultrastructural level using antisera against entire or alkaline dissolved polyhedra. It was possible to localize polyhedrin antigens in E. scandens larvae tissues and L. dispar cells in vitro but only from the beginning of the cristallization to the final maturation.

R29.24

DNA AND PROTEIN SYNTHESIS IN A PERSISTENTLY INFECTED SPODOPTERA FRUGIPERDA (SF-P) CELL LINE. W.C. Rice, A.H. McIntosh, C.N. Ignoffo. USDA, ARS, BCIRL, Columbia, MO, USA

Using viral specific probes, DNA:DNA dot blot hybridization and ^{35}S-Met pulse labeling experiments were used to analyze intracellular DNA and protein synthesis at various times in SF-P cells, and SF-P cells superinfected with either a homologous baculovirus (SfMNPV-OH) or a heterologous baculovirus (AcMNPV-L1). Such experiments revealed: the presence of a constant low level of SfMNPV-OH DNA synthesis in SF-P cells and confirmed its absence in S. frugiperda cells; normal DNA synthesis kinetics of S. frugiperda cells infected with either SfMNPV-OH or AcMNPV-L1; a delay in the onset of AcMNPV-L1 DNA synthesis in SF-P cells; and, an altered pattern of SfMNPV-OH DNA synthesis in SF-P cells, when compared to S. frugiperda cells, both in terms of time (earlier) and amounts (decreased). S. frugiperda cells infected with either AcMNPV-L1 or SfMNPV-OH showed the normal temporal pattern of expression of viral polypeptides. S. frugiperda and SF-P autoradiograms revealed that no polypeptides were unique to SF-P while Coomassie stain gels indicated that several larger Mw polypeptides present in both cell lines were stabilized in SF-P. SF-P cells infected with AcMNPV-L1 reveal a normal pattern of polypeptide synthesis relative to S. frugiperda cells while the pattern observed in SF-P infected with SfMNPV-OH is identical to that of SF-P.

R29.25

PRODUCTION IN E. COLI OF BMCPV POLYHEDRIN, A UNIQUE VIRAL PROTEIN THAT FORMS CRYSTALLINE OCCLUSION BODIES. C. Lavallée[1,2], M. Arella[1], S. Belloncik[1], Y. Furuichi[2] Institut Armand-Frappier, University of Québec, Montréal, Canada[1], Department of Molecular Genetics, Nippon Roche Research Center, Kamakura, Japan[2]

BmCPV polyhedrin was synthesized in E. coli transformed with an expression vector harboring the cloned polyhedrin gene. The yield was as high as 20% of total E. coli protein and allowed the preparation of polyhedrin in a large quantity. N-terminal amino acid sequencing and immunoblotting experiments confirmed that the polyhedrin produced in E. coli is identical to that obtained from virus infected Bombyx mori larvae. Polyhedrin, however, accumulated in E. coli cells in spherical inclusion bodies rather than as cubic crystals, as occurs in virus infected Bombyx mori cells. The E. coli derived polyhedrin was purified almost to homogeneity. It is soluble under alkaline conditions, similar to native polyhedrin, but easily precipitates to form aggregates under neutral pH. Strikingly, polyhedrin within the spherical inclusion bodies is rapidly degraded into small polypeptides when incubated with BmCPV polyhedra in alkaline buffer. Investigations concerning two unique structural features of polyhedrin, i.e. formation of rigid crystal polyhedra and sensitivity to CPV-associated protease, are under way using purified recombinant polyhedrin.

R29.26

Characteristics of integration regions of NPV genome and Prague stock Galleria mellonella (Gm) cell genome. I.N.Skuratovskaya, N.YU.Miryuta, L.I.Strokovskaya, I.P.Kok.Institute of Molecular Biology and Genetics, Kiev, USSR.

We showed earlier (1985) that Gm genome contained 2.0±0.6 copies of NPV genome per cell. The tests on reassociation kinetics and blot-hybridization of the thermal fractions of Gm cell DNA with different size of fragment with probes ^{32}P-labelled Gm NPV DNA have shown the link between viral and cellular DNA sequences. The blot-hybridisation experiments of viral and cellular DNA with ^{32}P-labelled unique and repeated Gm DNA sequences and the latter after S_1-nuclease treatment as probes have shown that repeated sequences are located in 4 or 5 regions of viral genome and amount to 3% of viral genome size.

On the basis of these data and Gm NPV genome physical mapping data the viral DNA integration into cell genome is suggested to be in NPV genome repeated sequences of the HindIII-B and -G-fragments. The cell DNA sequences which are included in integration are characterized by the higher GC-content than Gm NPV DNA one.

R29.27

A STUDY OF PROTEIN/DNA INTERACTIONS INVOLVED IN TEMPORAL REGULATION OF POLYHEDRIN GENE EXPRESSION IN AUTOGRAPHA CALIFORNICA NUCLEAR POLYHEDROSIS VIRUS. Vivienne Murphy and Martin Page, Wellcome Biotech, Beckenham, Kent, U.K.

A transient expression assay was used to demonstrate trans activation of the polyhedrin promoter by products of a virus infection. A plasmid containing the chloramphenicol acetyltransferase gene under control of the polyhedrin promoter was transfected using calcium phosphate precipitation into Spodoptera frugiperda (S.f.) cells. The gene was only expressed > 12 hours after infection of the transfected cells with Autographa californica nuclear polyhedrosis virus.

Specific protein/DNA interactions were studied using an electrophoretic mobility shift assay. End-labelled DNA fragments containing the N terminus of the polyhedrin gene and the promoter region were shown to form several specific complexes of reduced electrophoretic mobility in the presence of extracts from S.f. cells. Complexes of similar mobility were formed with extracts from uninfected cells and cells harvested 6 hours post-infection. Extracts from cells harvested 24 hours post-infection produced a single new stable complex, which was protected from DNAse digestion. The components of this complex were analysed by SDS/PAGE, and pulse-labelling experiments were used to trace the origin of the protein component. Specific sequences involved in the complex formation have been identified using a DNA footprinting assay with partially purified infected cell extracts.

R30.1

THE ROLE OF COXSACKIE B VIRUSES IN THE PATHOGENESIS OF MYOCARDITIS, DILATED CARDIOMYOPATHY AND INFLAMMATORY MUSCLE DISEASE, N.E. Bowles, L.C. Archard, Biochemistry Department, Charing Cross & Westminster Medical School, London, W6 8RF, UK

Coxsackie B viruses have been associated with a variety of myosites, particularly myocarditis and epidemic pleurodynia. However, the associations are derived from retrospective serology rather than direct virus isolation or immunofluorescent detections of viral antigens in the affected tissue. We have synthesized cDNA's and used them in molecular hybridisations to quantitative slot-blots of RNA prepared from relevant biopsies.

Of 50 patients studied with histologically proven myocarditis or dilated cardiomyopathy 28 (56%) were positive for Coxsackie B virus-specific RNA sequences. All 22 patients with other cardiac disease of known aetiology unrelated to virus infection were negative. Multiple biopsies had been obtained from 20 patients and of these 15 (75%) had at least one biopsy positive, indicating the focal nature of the disease.

In analogous experiments Coxsackie B virus RNA was detected in more than half of the biopsies tested from patients with juvenile dermatomyositis or adult polymyositis. Ten biopsies from normal or pathological controls were all negative. This is the first consistent demonstration of persistent Coxsackie B virus RNA in the affected tissue of patients with inflammatory muscle diseases.

R30.2

GENOMIC HOMOLOGIES IN THE PICORNAVIRIDAE FAMILY. W. Al-Nakib[1], M. Forsyth[1], G. Stanway[2], P. J. Hughes[2], J. W. Almond[3] and G. W. Leckie[3]. MRC Common Cold Unit, Salisbury[1], and the Departments of Biology, University of Essex[2], and Microbiology, University of Reading[3], U.K.

We have recently developed a cDNA:RNA hybridization system for the detection of rhinovirus RNA. We have prepared probes comprising sequences from both the 3' and 5' end regions of the genomes of human rhinovirus 14 (HRV-14), 1B (HRV-1B), 9 (HRV-9) and the 5' end of the genome of Coxsackie A21 virus. We have used these probes to study the genomic relationship between members of the picornaviruses (56 rhinoviruses and 30 enteroviruses). A distinct and interesting picture has emerged which suggests that certain rhinoviruses are very closely related while others are clearly more divergent. These relationships are different from those shown by antigenic studies. Furthermore, some rhinoviruses appear to be more closely related to some of the enteroviruses than to other rhinoviruses. In addition, the 3' end probes of HRV-14 and 1B appear to detect only the homologous serotype while the 5' end probes, which comprise mainly the non-coding region, have generally reacted with over 90% of rhinoviruses investigated.

These and other data to be presented have provided new and important information on the molecular relationships between members of the picornaviridae family.

R30.3

cRNA PROBES IN DETECTION AND CHARACTERIZATION OF PICORNAVIRUSES.

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We have developed a rapid and sensitive method for enterovirus detection using subgenomic RNAs synthetized in vitro as probes in molecular hybridization. This procedure allows to detect and partially characterize enteroviruses, not only in cell lysates but in crude clinical and environmental samples, like stools and sewage.

In addition, hybridization of various subgenomic poliovirus riboprobes representing variable (VP1) or conserved sequences (5'-non coding , VP3, 2C) with several picornaviruses allowed to establish the genomic relatedness between polio and some other viruses.

R30.4

A COMPARISON OF 10 DIFFERENT CLONED BLUETONGUE VIRUS GENOME SEGMENTS AS PROBES FOR THE DETECTION OF VIRUS-SPECIFIC RNA. H. Huismans, M. Cloete. Biochemistry Section, Onderstepoort Veterinary Research Institute, South Africa.

Full-sized cloned DNA copies of the 10 double-stranded RNA genome segments of bluetongue virus (BTV) were used as ^{32}P-labeled probes for the detection of virus-specified RNA from 24 different BTV serotypes. It was found that genome segment 2, which codes for outer capsid protein P2 was serotype-specific. Cross hybridization of segment 2 accurately reflected cross neutralization that is observed. Genome segment 6, which codes for outer capsid protein P5, was almost equally serotype-specific but there is no evidence that P5 is involved in determining a neutralization-specific immune response. Genome segment S7 which codes for the group-specific antigen P7 on the surface of the core particles of BTV is also not very highly conserved. Capsid proteins P1, P3 and P4 are probably the most highly conserved and would be suitable candidates for a group-specific probe. The most highly conserved gene of BTV is, however, the one that codes for the major non-structural protein of BTV, NS1. This protein is the major component of the characteristic tubules found in BTV-infected cells. No cross hybridization was observed of the BTV probes with closely related orbiviruses. More sensitive high specific activity RNA probes of BTV genes did, however, cross hybridize with Epizootic hemorrhagic disease virus RNA.

R30.5

MONOCLONAL ANTIBODY HYBRIDIZATION FOR THE DETECTION OF RNA VIRUSES. Raphael Viscidi and Robert Yolken. Johns Hopkins School of Medicine, Balto., Md.

Nucleic acid hybridization is an efficient means for the detection of viral nucleic acids. However, standard hybridization protocols can require extensive time and manipulations. We have developed a rapid method of nucleic acid hybridization which utilizes biotin-labelled probes and monoclonal antibodies directed at DNA-RNA hybrids. Biotinylated c-DNA probe complementary to the target viral RNA is incubated with the sample. Hybrids are subsequently isolated from the liquid phase by binding to solid phase anti-biotin and identified by reaction with B-galactosidase labelled monoclonal antibody directed at DNA-RNA complexes and fluorescent enzyme substrate. The reactions can be completed in less than 4 hours and are not effected by contaminating bacteria or interfering factors present in human body fluids. We have applied this system to the detection of a wide range of RNA viruses including rotaviruses, picornaviruses and human immunodeficiency virus. Monoclonal antibody hybridization offers the potential for being a practical technique for the rapid diagnosis of viral infections in humans.

R30.6

DOT HYBRIDIZATION ASSAY FOR THE DETECTION OF ROTAVIRUSES IN CLINICAL SPECIMENS: SPECIFICITY OF THE ASSAY IN THE PRESENCE OF RIBOSOMAL RNA. S. Sato R. Yolken, J. Eiden. Department of Pediatrics, Johns Hopkins University School of Medicine, Baltimore, Maryland.

Caution has been advised in the use of nucleic acid probes for the detection of RNA viruses in clinical specimens, because several viral RNA genomes have been reported to hybridize to mammalian rRNA under stringent conditions (J Virol 1986; 57: 917). Therefore, we assessed the affect of rRNA on the specificity of a diagnostic dot hybridization assay for the detection of rotaviruses (RV). Eucaryotic rRNA was demonstrated in phenol-extracted fecal specimens from 54 of 95 patients. A sensitive immunoassay for RV was non-reactive in 82/95 specimens, and 81 of these 82 were also non-reactive by dot hybridization (specificity=98.8%). Of the 13 specimens reactive in the RV immunoassay, 8 were also reactive by dot hybridization (sensitivity=61.5%). The predictive value of a positive RV dot hybridization was 88.9%, and the predictive value of a negative test was 94.2. These data indicate that the presence of eucaryotic rRNA in clinical specimens does not affect the specificity of a diagnostic dot hybridization for rotaviruses.

R30.7

DETECTION OF VARICELLA VIRUS DNA IN INFECTED CELLS WITH DNA HYBRIDIZATION TECHNIQUE. G. Cuthbertson, C. Grose. Department of Pediatrics, Faculty of Pediatric Infectious Disease, University of Iowa, Iowa City, Iowa, U.S.A.

DNA hybridization techniques have been used successfully in various studies to detect viral DNA from infected cells. We have adapted a method used primarily with the cytomegalovirus (CMV) genome to allow detection of varicella virus (VZV) DNA in infected human cells. Monolayer cultures of "Hewo" strain of human melanoma cells (HMC) infected with VZV-32 virus stock were harvested at 12, 24, 36, and 48 hours postinfection. Controls included uninfected HMC monolayers. A standard phenol extraction method was used to isolate the cellular DNA, which was then quantified, serially diluted, and blotted onto nitrocellulose membranes secured in a "Bio-dot" apparatus. The DNA probe was produced in a nick translation enzyme system containing 100 microcuries ^{32}P and 0.4 micrograms (µg) HindIII fragment A of the varicella genome. Blotted membranes were placed in this probe solution at 68°C for 48 hours.

Exposure on x-ray film showed a dot with varying intensity whenever viral genome was detected. We have detected 0.5 µg total DNA from cells incubated 12 hours postinfection and 0.1 µg after 48 hours. All unifected cell samples were negative. We have also used this hybridization method to detect VZV DNA in tissue samples from patients with chickenpox or zoster.

R30.8

PRODUCTION OF BIOTINYLATED PROBES TO BOVINE CORONAVIRUS. J.A. Verbeek, P. Tijssen, Centre de recherche en médecine comparée, Institut Armand-Frappier, Université du Québec, Montréal, Québec, Canada.

Bovine coronavirus was highly efficiently propagated and isolated from tissue culture cells. Genomic viral RNA-molecules were isolated and subsequently reverse-transcribed using both oligo dT- and oligo dT- plus random primers. First-strand cDNA/RNA heteroduplex molecules were incubated with RNase-H to produce RNA primers on the cDNA for the synthesis of double-stranded cDNA molecules by means of Klenow polymerase. These molecules were dC-tailed and cloned into a Pst-I digested dG-tailed- pUC-9 vector. Depending on the primers used, two sets of clones have been produced; those specifically starting from the 3' end of the viral RNA and those presenting a random distribution of parts of the whole virus genome. Inserts of interest were subsequently subcloned into bacteriophage vector M13 (mp18 and mp19) and sequenced. Clones with specific sequences (genes/gene fragments) were amplified, purified and biotinylated to produce virus specific probes. Biotinylated markers were used due to their long term stability and sensitivity in detection methods. Further analysis was done on the different hybridization conditions under which these probes are capable to specifically detect coronavirus in fecal-samples and in tissue culture grown virus.

R30.9

DETECTION OF ENTEROVIRUSES IN WATER USING GENE PROBES. A. Margolin, C.P. Gerba. Dept. of Microbiology, University of Arizona, Tucson, USA.

Current methods for the detection of enteroviruses in water requires the use of animal cell culture. This technique has several drawbacks, such as: 1) long incubation periods, 2) not all viruses can be detected in one cell line, and 3) not all viruses have been grown in tissue culture. More rapid techniques, such as fluorescent antibody or radioimmunoassay do not have the needed sensitivity to detect the low levels of viruses found in contaminated water. Recent advances in recombinant DNA technology now make it possible to detect viruses without the use of cell culture or antibodies. Gene probes that hybridize to the RNA of poliovirus and hepatitis A virus were tested for their ability to detect different enteroviruses. Upon comparison to cell culture, the dot-blot assay was as sensitive as tissue culture for the detection of poliovirus in environmental samples such as secondary effluent and tapwater.

R30.10

THE DETECTION OF INFECTIOUS ENTEROVIRUSES USING cDNA PROBES. B. Trumper, A. Margolin, C.P. Gerba. Department of Microbiology, University of Arizona Tucson, USA.

Recently, gene probes to enteroviruses have been used in diagnostic tests to determine the presence of viral nucleic acids in concentrated water samples. While these tests are able to indicate that viruses are present, they do not differentiate between infectious and non-infectious viruses. To determine this, monolayers of BGM cells were inoculated with poliovirus, then lysed, treated with Protease K, centrifuged, and applied to gene screen plus hybridization membrane. Using the cDNA probe to poliovirus, preliminary results indicated infectious virus could be detected 20 hrs post-adsorption. Using this assay system, environmental water samples may be screened for the presence of infectious viruses.

R30.11

COMPARISON OF BIOTIN PROBES, RADIOACTIVE PROBES, AND TISSUE CULTURE FOR THE DETECTION OF ENTEROVIRUSES. Kenneth J. Richardson, A.B. Margolin, C.P. Gerba. Dept. of Microbiology, University of Arizona, Tucson, USA.

Radioactive nucleotides traditionally have been used in labling gene probes. The development of non-radioactive lables such as biotin, has several advantages including reduced hazards, decreased cost, and a longer shelf life.

A biotin-11-dUTP analog was used in a nick translation reaction to lable a poliovirus cDNA probe. To increase the sensitivity of the probe, the labled cDNA fragments were subjected to poly T homopolymer tailing using terminal transferase. For the detection of femptogram quantities of viral nucleic acid, the probe was hybridized to the target RNA followed by a second hybridization of a biotin labled poly A fragment.

Preliminary results indicate that the sensitivity of this probe is one to two logs less sensitive than radioactive labled probes and tissue culture assays. Currently, our lab uses ^{32}P labled gene probes for the detection of enteroviruses in a concentrated water sample. This research will indicate if biotin probes can replace radioactive probes as an assay system.

R30.12

PORCINE PARVOVIRUS (PPV)-SPECIFIC AND GENERAL PARVOVIRUS DNA PROBES. J. Bergeron, J. Menezes and P. Tijssen. Institut Armand-Frappier, Université du Québec, Laval, Québec Canada, H7N 4Z3. Hôpital Ste-Justine, Université de Montréal, Montréal, Québec.

Porcine parvovirus (PPV) was successfully adapted to Porcine Fallopian tube cells (PFT) and the double-stranded replicative form (RF) DNA was directly obtained from infected cells using the Hirt extraction method. Restriction enzyme analysis revealed a slight difference with published restriction maps. The large Eco RI-Pst I fragment (3.3kbp) was cloned into pUC 18, followed by subcloning to obtain smaller fragments of the different parts of the genome. The Eco RI-Pst I fragment was also transferred from pUC 18 to M13 mp18/19 for sequencing by the Sanger method. Sequence analysis revealed a particular 0.15 kbp sequence (HC fragment) at 37 map units which is highly-conserved amoung parvoviruses. Clones each containing a different fragment, including HC, were propagated, purified and biotin-labeled by nick translation. The optimal hybridization conditions as well as the usefulness of the different probes for the detection of PPV in different samples was established. The applicability of the HC probe for the detection of parvoviruses from other animals was investigated. Moreover, a new experimental design of probing was developed which allowed an increase in the detectability of a least 10 times.

R30.13

DETECTION OF BLUETONGUE VIRUS AND BOVINE HERPESVIRUS-1 NUCLEIC ACIDS IN CELLS AND TISSUE SECTIONS USING BIOTINYLATED HYBRIDIZATION PROBES. R. Schoepp, B. Beaty, C. Blair, J. Collins. Department of Microbiology, Colorado State University, Fort Collins, Colorado, USA; P. Roy, Department of Public Health, University of Alabama-Birmingham, Birmingham, Alabama, USA.

Sensitive molecular hybridization techniques have been developed to detect bluetongue and bovine herpesvirus-1 nucleic acids in situ in cell cultures and in formalin-fixed, paraffin-embedded tissue sections. DNA probes were biotin labeled by nick translation, and the presence of hybrids were revealed using either horseradish peroxidase or alkaline phosphatase reporter systems. Optimal conditions for cell fixation, permeation, post-fixation for retention of analyte, and detection were determined.

BHV-1 nucleic acids were detected 2 hrs post infection in the cytoplasm of infected cells. Signal was first detected in the nucleus 4 hrs post infection. By 15 hrs, both cytoplasm and nucleus of 95% of the cells were infected. Treatment with ribonuclease or deoxyribonuclease before hybridization allowed differentiation of virus specified DNA and RNA within the cells. BHV-1 nucleic acids were detected in nasal epithilial cells obtained from inoculated calves.

Similar studies with BTV nucleic acid detection will be reported.

Molecular hybridization techniques are being compared with newly developed monoclonal antibody antigen detection techniques for detection of virus in cells and tissue sections in clinical trials at the Diagnostic Laboratory of the Colorado State University Veterinary Teaching Hospital.

R30.14

A SIMPLE SENSITIVE TECHNIQUE USEFUL FOR INVESTIGATIONS ON ENVELOPED VIRUSES. E. N. Arida Cheif, Laboratory of Viruses & T.C., National Research Center, Dokki, Cairo, Egypt.

A simple multistep technique was developed for investigations on enveloped viruses. It was suitable with purified virus preparations, virus infected cells, biological fluids, tissues, arthropods, etc,... It is based on extraction of viral cores from an aqueous phase containing traces of a nonionic detergent, into a pretreated light organic solvent phase at 0-4 C. The hydrophobic character of the lipid coated core surface allows the envelop protein-deprived cores to be selectively extracted into the light organic solvent.

The extracted viral cores can be used in infectivity studies. RNA in disrupted cores prepared from RNA viruses proved to be biologically active in all cases. It was translated in eukaryotic & prokaryotic cell free systems. The polypeptides formed had immunological & electrophoretic properties identical to those of the starting virus. Core proteins were characterized by ELISA, immunoperoxidase technique, reverse transcriptase inhibition assays, etc,.. This technique was useful for investigations on myxoviruses, retroviruses, togaviruses, etc,...

R30.15

Nonradioactive labelling and detection of nucleic acid probes

R Seidl, J Holtke, C Kessler, P Matles

Boehringer Mannheim GmbH Tutzing FRG

We present a system for nonradioactive labelling hybridisation and detection with comparable sensitivity to radioactivity, which gives not only in spot hybridization tests but with complex material comparable results. None of the commercial kits avalaible at the time can fulfill these criteria.

With our protocol we can detect a single copy gene after digestion and blotting of down to less than 0.5 pg genomic DNA in spot hybridisation tests (diluted plasmid versus labelled plasmid) we reached a sensitivity of at least 0.1 pg DNA. These results were obtained with biotin-streptavidin. The DNA-probes were labelled enzymatically by nick translation or random primed labelling using modified nucleotides (biotin). Hybridization and post-hybridization washing was performed according to standard procedures. After blocking nonspecific binding the filters were probed with a streptavidin-alkaline phosphatase conjugate.

R30.16

RAPID DIAGNOSIS OF CYTOMEGALOVIRUS INFECTION BY DETECTION OF CYTOMEGALOVIRUS INDUCED EARLY ANTIGENS OR DNA SEQUENCES IN SHORT TERM CULTURES. H.P. Janssen*, W.G.V. Quint**, M.J.M. Meddens**, P. Herbrink** J.Lindeman** and A.M.v.Loon*. *)Dept. of Medical Microbiology, Univ. of Nijmegen, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands. **)SSDZ, Dept. of Pathology, P.O. Box 5010, 2600 GA Delft, The Netherlands.

To examine the suitability for rapid diagnosis of cytomegaovirus (CMV) infection, we studied the sensitivity of early antigen (EA) detection and in situ DNA hybridization at various time points and concentrations after inoculation of tissue culture cells with CMV strain AD 169.

Immunological detection of CMV-EA was the most sensitive method during the first three days after inoculation (sensitivity $\pm 10^2$ TCID 50/ml). Starting at day six after inoculation, the sensitivity of CMV-EA detection was similar to that of CMV-DNA detection and conventional virus isolation. Therefore, these results in a model system indicate that it might be possible to replace conventional virus isolation procedures (which may take up to six weeks) by either detection of CMV-EA or CMV-DNA after short term culturing. A study confirming these results in patients' material from an ongoing renal transplant CMV screening program will be presented.

R30.17

PLASMID LIBRARY FOR IN VITRO TRANSCRIPTION OF SPECIFIC REGIONS SPANNING THE ENTIRE HTLV-III GENOME. C. Roy, P. Garvie, B. Linner and J. Camplone-Piccardo. Molecular Virology Laboratory, Diagnostic Reagents Division, Laboratory Centre for Disease Control, Health and Welfare Canada, Ottawa, Ontario K1A 0L2 Canada

The most commonly used plasmid for the synthesis of ribonucleic acid (RNA) probe complementary to the genome of human immunodeficiency virus (HIV-1) is a pSP64-based plasmid containing the SstI 8.9 Kb fragment of strain HTLV-III (human T-lymphotropic retrovirus type III). The in vitro transcripts obtained from this template are usually 2 Kb long and they predominantly span the 3' end ORF and the ENV (gp41) regions in the viral genome. We have rearranged the HTLV-III sequences with respect to the SP6 promoter in pSP64 and have obtained a series of new constructs allowing the expression in vitro of RNA transcripts complementary to other regions of the viral genome. This library allows the production of in vitro transcripts specific for the 5' end of the ENV region (gp120), the TAT (SOR), the POL and the GAG regions. The combined use of this library allows the production of RNA probes complementary to the entire HTLV-III genome and at template saturation conditions they result in a several fold increase in the sensitivity of hybridization procedures. The use of these probes in the analysis of DNA and RNA of infected T4 lymphocytes will be discussed. The authors wish to thank Drs. M. Harper, R. Gallo and F. Wong-Staal (NIH) for providing plasmid pBH10R3.

R30.18

RAPID DIAGNOSIS OF HERPES- AND ADENOVIRAL INFECTIONS BY DIRECT FILTER HYBRIDIZATION. S. Belák*, T. Linné*, A. Bartha**, M. Benkö**, G. Rockborn*, M. Rusvai***. *Virol. SVA/SLU, Biomedical Center, Uppsala, Sweden, **Vet. Res. Inst. Acad. Sci. and ***Dept. of Epizootiol., Univ. Vet. Sci., Budapest, Hungary.

Aujeszky's disease (AD), infectious bovine rhinotracheitis (IBR) and adenoviral infections were detected by a rapid direct filter hybridization method, in which there is no need for purification of DNA from the specimens. Virus specific DNA clones corresponding to fragments of pseudorabies, IBR and bovine adenovirus types 2 and 4 were used as probes.

The method was tested on cultured infected cells, on experimentally infected pigs (AD), calves (IBR, adeno) and lambs (adeno) and on specimens from an outbreak of AD. The analysis of clinical specimens showed concordance with virus isolation and with hybridization of purified DNA, indicating that the direct filter hybridization method is a good tool of rapid diagnosis. The further advantages of the method are (a) it is independent of cell culture facilities; (b) an easy and fast sample processing; (c) simplified hybridization procedure; (d) the diseases are diagnosed within 15 hours.

R30.19

SCREENING OF CERVICAL PRENALIGNANT LESIONS FOR HPV TYPE 16 DNA BY SANDWICH AND IN SITU HYBRIDIZATION METHODS

N. Ranki[1], S. Parkkinen[2], K. Syrjänen[2] and S. Syrjänen[2]
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2) University of Kuopio, Kuopio, Finland

Cervical scrapes (97) were obtained from women with suspected papillomavirus infection and screened for HPV 16 by the sandwich (N. Ranki et al. Gene (1983) 21, 77) and in situ hybridization methods. The number of cells obtained in the scrapes was also determined. Both methods detected 22/97 HPV 16 positive scrapes. 18 additional cases were detected by the in situ and 7 by the sandwich method only. Most of the discrepant results were explained by an inadequate number of cells recovered for the sandwich assay, or a technical error not permitting study by the in situ method. Some of specimens contained HPV 33, being detected by the HPV 16 probe in the in situ test but not in the sandwich test. The positivity of the scrapes correlated highly significantly with the CIN and PAP grades III and IV.

The advantages of the sandwich method are an easy pre treatment of the specimen (boiling in SDS) and background free test that can be completed in 24 h. The study shows that the sandwich technique can be used as a diagnostic method to screen cervical scrapes and biopsies for HPV 16 DNA.

R30.21

GENERAL AND STRAIN-SPECIFIC GENOMIC PROBES FOR INFECTIOUS BURSAL DISEASE VIRUS.

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Department of Biochemistry and College of Veterinary Medicine, Mississippi State University, Miss. State, USA.

Infectious bursal disease virus of chickens is a nonenveloped 60-nm particle containing a genome of two double-stranded RNA segments about 3400 bp and 2900 bp long, respectively. To facilitate both the epidemiological study of the disease and the development of a subunit vaccine, a series of genomic probes have been developed.

Virus was propagated in vivo and in vitro, both in primary chicken and transformed mammalian cell cultures. Initially, general ^{32}P-labelled probes detecting all tested virus strains, of both vaccine and field origin, were generated using the entire RNA genome as template for formation of randomly-primed cDNA's. Then probes discriminating among strains were made. Separate genomic cDNA libraries of several strains were assembled in order to clone sequences with the requisite autologous strain homology and heterologous strain non-homology.

All probe protocols were adapted to screen dot blots of preparative tissue homogenates for presence of the viral genome. Over many trials, the minimum time required between procurement of suspect tissue and determination of probe results was consistently less than 36 hours.

R30.20

IDENTIFICATION OF CHANNEL CATFISH VIRUS USING A NUCLEIC ACID PROBE.

J. Boyle, J. Wise, S. Harrell, P. Bowser.
Department of Biochemistry, Mississippi State University, Mississippi State, USA.

Channel catfish virus (CCV) is a herpesvirus that can cause high mortality among fingerling and fry catfish. Adult fish can apparently harbor the virus with no obvious symptoms. In these adult fish the virus is routinely detectable only by using a recently developed recombinant DNA probe. The virus is generally present in the adult fish in very low numbers and in various tissues.

The transmission of CCV has been studied using the DNA probe. Adults carrying CCV in an asymptomatic state were mated and the resulting fertilized eggs were isolated from further contact with the fish. Offspring from this mating were found to contain the virus. This indicates some form of vertical transmission.

The state of the CCV DNA in the adult fish was also examined to help elucidate more fully the exact mechanism of this transmission and the nature of the viral latency. Clear evidence of an alteration in the viral DNA was observed.

R31.1

EPIDEMIOLOGY OF TOMATO SPOTTED WILT VIRUS INFECTING GROUNDNUT IN MAHARASHTRA. *D.D. Nirmal, V.R. Mali, Department of Plant Pathology, Marathwada Agricultural University, Parbhani, Maharashtra, India.*

Groundnut bud necrosis disease (BND) caused by tomato spotted wilt virus (TSWV) afflicted losses ranging from 1-100% depending upon age, type of cultivar and season. BND is prevalent in groundnut growing districts of Maharashtra though incidence is found more in Kharif (rainy) (July to Oct.) than Summer (Feb. to May) season; the vulnerable stage being a seedling stage (30-45 days after sowing). Frankliniella schultzei and Scirtothrips dorsalis transmitted TSWV in a circulative, non-propagative manner; the former being the most efficient vector and mainly responsible for BND field spread. TSWV also infected spontaneously hosts like tomato, brinjal, cowpea, mungbean, urdbean, Alysicarpus rugosus, Calotropis procera, Desmodium dichtomum and Solanum nigrum. Many of these were also hosts of thrips vectors. Incoming viruliferous thrips caused primary infections and disease incidence being positively correlated with thrips population dynamics. The secondary infection occurring within field is non-significant and BND spreads in the field with a pattern of simple interest disease. Wind velocity and temperature are critical in epidemiology of groundnut TSWV.

R31.2

CONTROL OF A NONPERSISTENT PLANT VIRUS BY SYNTHETIC PYRETHROIDS

G.I. Atiri. Department of Agricultural Biology, University of Ibadan, Ibadan, Nigeria.

The efficacy of the newer synthetic pyrethroids such as cypermethrin and deltamethrin in reducing transmission of the nonpersistently transmitted cowpea aphid-borne mosaic virus (CAMV) in cowpea was compared to insecticides such as a carbamate (pirimicarb) and an organophosphate (dimethoate).

The number and duration of aphid probe was lower on pyrethroid-treated than on pirimicarb-and dimethoate-treated plants, indicating that aphids did not probably probe long enough to transmit CAMV. In the field, the rate of spread of CAMV was slowest, and yield responses were generally highest for treatments with pyrethroids.

It is concluded that the newer synthetic pyrethroids have the potential to reduce the spread of seemingly intractable nonpersistent viruses, and further studies should be conducted to determine how their use can best be incorporated into farming practices.

R31.3

INFECTION PATHWAYS OF PLANT RHABDOVIRUSES FOR INSECT VECTOR CELLS. K. Gaedigk-Nitschko, C. Stussi-Garaud, K.-W. Mundry and G. Adam. Inst. for Biology, University of Stuttgart, Dept. of Botany, F.R.G. and IBMC du C.N.R.S., Strasbourg, France.

Plant rhabdoviruses are distributed in nature by insect vectors which must become infected before they can transmit the virus. In order to understand the mechanism of this circulative-propagative transmission mode, knowledge of the infection pathway for the vector cells is important.

We have studied the pathway of infection with the rhabdovirus potato yellow dwarf and an established vector cell line from the cicadellide vector Aceratagallia sanguinolenta, using both, biochemical methods and the electronmicroscope.

The amount of virus adsorbed to the cell surface increased during 300 min without reaching a plateau. Virus particles adsorbed could be detected by electronmicroscopy. Uptake of adsorbed virus into the cells, leading to infection, was rapid. After 30 min at 28°C the number of infected cells could no longer be reduced by treatment with proteases. Inhibition of infection by lysosomotropic substances was indicative for an endocytotic pathway with fusion inside of the cells. However, under conditions of total inhibition of endocytosis adsorbed virus could infect cells when the pH was decreased below 5.6. The pH-dependence of this type of infection indicated that direct fusion of virus with the outer cell membrane was another possible infection pathway.

R31.4

THE ROLE OF ANTI-IDIOTYPIC ANTIBODIES IN STUDY OF BARLEY YELLOW DWARF LUTEOVIRUSES. J. S. Hu, W. F. Rochow, and H. W. Israel. Department of Plant Pathology, Cornell University, Ithaca, NY, and F.E. Gildow, The Pennsylvania State University, University Park, PA, USA.

Anti-idiotypic antibodies (ABs) were produced in rabbits against an anti-RPV monoclonal AB (mAB-RPV1), which neutralizes RPV transmission by *Rhopalosiphum padi*. In three previous lines of work, we found the ABs to be true anti-idiotypic ABs. When the anti-idiotypic ABs were injected into a rabbit, active anti-RPV ABs were produced. Production of the anti-anti-idiotypic ABs not only provided additional evidence for the nature of the anti-idiotypic ABs, but also suggested a new approach for the production of ABs against hard-to-obtain plant viruses. In bioassays to date, aphids injected with the anti-idiotypic ABs transmitted RPV normally. Progress has been made in electron microscope work using the anti-idiotypic ABs to study putative virus receptors on aphid salivary gland membranes. Potential applications of this technique in plant virology will be discussed.

R31.5

CLONING AND EXPRESSION OF THE POTYVIRUS HELPER COMPONENT GENE. Y. Stram, P. H. Berger, T. P. Pirone. Department of Plant Pathology, University of Kentucky, Lexington, Kentucky.

A 4.2 KB cDNA sub-clone of tobacco vein mottling virus (TVMV) RNA containing the gene coding for TVMV helper component (TVMV-HC) was trimmed by restriction enzyme digestion to give a 1.5 KB fragment which contained the entire putative HC gene. The construct also contained additional terminal nucleotides which would result in the presence of an extra 22 amino acids at the putative amino terminus and 3 extra at the carboxy terminus of a polypeptide product of the construct. A 10-mer oligodeoxynucleotide that contained an ATG in the correct translation frame was added to the 5' end of the cDNA prior to cloning into an E. coli expression vector. Analysis of the product from induced, transformed E. coli cells on western blots using TVMV-HC antiserum revealed two major products that reacted with the antiserum, one about 2 KD larger than authentic TVMV-HC and the other about 8 KD smaller. Bioassays of the E. coli product failed to show HC activity. The cDNA construct of the TVMV-HC gene was also used with the agrobacterium Ti plasmid system to transform tobacco cells. Western blot analysis revealed that there is a TVMV-HC related polypeptide in transformed tobacco cells that is 10 KD larger than helper component from TVMV infected plants.

R31.6

THE EFFICIENCY OF VECTOR TRANSMISSION OF NON-PERSISTENTLY TRANSMITTED VIRUSES IN THE FIELD. D. Peters and T. Terwisscha van Scheltinga. Agricultural University, Department of Virology, Wageningen, The Netherlands.

The efficiency of virus transmission by aphids is usually studied in laboratory experiments in which the aphids are confined for fixed periods on the source and test plant. Since the aphids are usually strongly hindered in their mobility in such experiments, the efficiency found may not correspond with that in the field. In studies, in which different techniques were used, the efficiencies found differed with the technique, the aphid species and the developmental stage of the used. Transmission of potato virus Y from and to Physalis floridana was much more efficient by Acyrthosiphon pisum than by Myzus persicae in an experimental set up to simulate field conditions. These and other results show that models which describe the spread of viruses in the field can only be developed when the efficiency of virus transmission by those developmental stages which contribute to this spread, has been measured.

R31.7

DEMONSTRATION OF LONG-TERM ASSOCIATION OF TOBACCO STUNT VIRUS (TSV) WITH OLPIDIUM BRASSICAE RESTING SPORES AND THE PRESENCE OF VIRAL GENOME IN ZOOSPORES.

C. Hiruki and S.A. Nasri, Department of Plant Science, University of Alberta, Edmonton, Alberta T6G 2P5, Canada.

TSV was recovered from resting spores that had been air-dried for 20 years in roots of tobacco (Nicotiana tabacum L. cv. Bright Yellow) by using Bright Yellow tobacco and tomato (Lycopersicon esculentum Mill. cv. Earliana) as bait plants. The anti-TSV rabbit-globulin prepared by administering TSV immunogen from tobacco leaves reacted specifically to TSV antigens from both tobacco and tomato up to 1/100 dilution of sap samples in double sandwich ELISA. Sap samples prepared from leaf tissues showing TSV symptoms contained rod-shaped particles measuring approximately 350 x 22 nm. When TSV-RNA of 4.2×10^6 daltons (5900 bp) was used as a cDNA probe, an identical genomic RNA was detected in zoospores of Olpidium brassicae isolated from TSV-infected tobacco plants, but was absent in those from healthy tobacco plants. In addition, a small molecular weight nucleic acid of about 0.4 K daltons was found in both TSV-carrying Olpidium as well as TSV-free Olpidium. This small nucleic acid was not found in TSV-RNA preparation.

R31.8

LOSS OF APHID TRANSMISSIBILITY IN THE CAMPBELL ISOLATE OF CAULIFLOWER MOSAIC VIRUS IS ASSOCIATED WITH A SINGLE AMINO ACID CHANGE IN ORF II. C.J. Woolston, C. Barker, P.G. Markham, A.S. Goad, R. Hull, J.W. Davies and A.J. Maule. John Innes Institute, Colney Lane, Norwich, U.K.

An antibody specific for p18, the protein product of CaMV ORF II implicated in the aphid-transmissibility of the virus, was used to detect p18 in tissue infected with an aphid-transmissible (AT+) isolate, Cabb B-JI. The protein was not detected in tissue infected with the aphid non-transmissible (AT-) CM4-184 isolate which contains a large deletion in ORF II. In contrast, using sensitive immuno-blotting methods p18 could be detected in tissue infected with the AT- Campbell isolate, indicating that the AT phenotype of the virus is not directly correlated with the presence or absence of the ORF II product.

The nucleotide sequence for the region of the Campbell genome implicated in the determination of aphid transmissibility was obtained and showed no obvious gross alterations relative to the wild-type sequences. The construction of in vitro recombinant hybrid genomes between Campbell and Cabb B-JI isolates clearly demonstrated that the loss of aphid transmissibility could be pinpointed to either or both of two base changes within the ORF II region. Only one of these base changes has an effect on the primary structure of the protein, glycine to arginine at amino acid position 94, and is thus the most likely cause of the alteration in AT phenotype.

R31.9

IN VITRO STUDY OF THE BIOLOGICAL ACTIVITY ASSOCIATED WITH THE APHID TRANSMISSION FACTOR OF CAULIMOVIRUSES. Ana M. Espinoza, P.G. Markham and R. Hull. John Innes Institute, Colney Lane, Norwich, U.K.

The interaction between the aphid vector and the transmitted entity of cauliflower mosaic virus (CaMV) is being investigated in fractions purified from turnip infected with isolate B-JI. An assay system using Myzus persicae was developed to enable crude extracts and purified fractions to be acquired in vitro. Successful transmission depended on optimising the molarity and pH of the buffer and the presence of chelating agents which affected the Ca^{++} and Mg^{++} balance. The assay system was sensitive enough to obtain transmission with single aphids; the in vitro transmission rate (47-70%) compared favourably to transmission from plant to plant (38-72%). The separation procedure showed that higher activity could be found in a fraction not containing the inclusion body. Results of infectivity obtained by mixing different fractions from the purification procedure will be discussed.

In vitro complementation of the helper component was demonstrated using CaMV and figwort mosaic virus.

R32.1

ANTIVIRAL NATURE OF SOME AMANTADINE DERIVATIVES. Arun K. Misra and Gloria L. Anderson. Morris Brown College, Atlanta University Center, Atlanta, GA 30314 USA.

Amantadine(trade name Symmetrel) is known to be effective against Influenza Virus A, Rubella Virus, and some oncogenic RNA viruses. We have synthesized and developed novel amantadine derivatives. We have also obtained some preliminary data on the inhibition of some microbes by different amnatadines. We have been testing the antiviral activities of various amantaidine derivatives(especially with Cl, F, I, NH_2 substitutions) against different viruses. Examination of virus-infected cells and tissues with electron microscope and the effects of amantadine treated cells with relation to virus infection will be explained. Results of testing the antiviral potential of 3-Fluoro-1-amino-admantane hydrochlori-de, 3-Chloro-1-aminoadnantane hydrochloride, N-(3-Fluoroadmantyl) urea, and 3-Iodo-1-Aminoadnantane hydrochloride, in comparison to ribavirin, against Influenza Viruses(especially AO and A2 types will be presented. MDCK cell tyne has been used to ascertain the cytotoxic effects of the amantadine derivatives: The recent trends in antiviral chemotherapy will be discussed and alternative approaches to attempt the control of human nathogenic viruses will be suggested.

R32.2

DIFFERENTIAL METABOLISM OF (E)-5-(2-IODOVINYL)-2'-DEOXYURIDINE (IVDU) BY EQUINE HERPESVIRUS TYPE 1- (EHV-1-) AND HERPES SIMPLEX VIRUS TYPES 1- AND 2- (HSV-1-, HSV-2-) INFECTED CELLS. S. Kit,[1] H. Ichimura,[1] E. De Clercq.[2] Division of Biochemical Virology, Baylor College of Medicine, Houston, U.S.A.,[1] and and Rega Institute, Katholieke University, Leuven, Belgium.[2]

Pyrimidine deoxyribonucleoside kinases (PyK) encoded by HSV-1, HSV-2, and EHV-1 catalyze the phosphorylation of dThd and BVDU. The replication of HSV-1 is sensitive to BVDU, but those of HSV-2 and EHV-1 are not. The differential sensitivity of the viruses to halogenated vinyldeoxyuridine drugs has now been investigated by studying the phosphorylation of [^{125}I]IVDU by enzymes from herpesvirus-infected, dThd kinase-negative RAB(BU) cells. Enzymes from cells infected by the 3 viruses phosphorylated [^{125}I]IVDU to IVDUMP, but only HSV-1-induced enzymes generated IVDUDP and IVDUTP. Experiments with mixtures of EHV-1- and HSV-1-induced enzymes showed that the EHV-1 enzyme did not inhibit IVDUDP and IVDUTP formation by the HSV-1 enzyme. With [^{125}I]IVDU as substrate, the Km values for EHV-1 and HSV-1 PyKs were 1.83 and 0.34 μM, respectively, and the Ki (dThd) value for the EHV-1 Pyk was 0.35 μM. In vive experiments confirmed that only HSV-1-infected cells converted IVDU to di- and triphosphates, indicating that inefficient phosphorylation of the monophosphates contributes to the insensitivity of EHV-1 replication to BVDU and IVDU, as it does to the insensitivity of HSV-2 replication to these drugs.

R32.3

TOLERANCE AND PROPHYLACTIC EFFICACY OF A NEW ANTIRHINOVIRUS COMPOUND, R61837. W. Al-Nakib[1], P. G. Higgins[1], D. A. J. Tyrrell[1], I. G. Barrow[1], N. Taylor[2] and K. Andries[2]. MRC Common Cold Unit, Salisbury, U.K.[1] and Janssen Pharmaceutica Beerse, Belgium[2].

This study describes the first double-blind placebo controlled clinical trials of a new antirhinovirus compound, R61837 or 3-methoxy-6-[4-(3-methylphenyl)-piperazinyl]-pyridazine in human volunteers challenged with human rhinovirus 9 (HRV-9).

R61837 was well tolerated when given as a nasal spray (a solution of 2.5 mg/ml) as none of the 16 volunteers who participated in the tolerance studies and who received either drug or placebo suffered upper respiratory symptoms or reaction.

Prophylaxis with R61837 (28 volunteers received drug and 27 received placebo) resulted in 5-fold reduction in the mean total daily clinical score and 3-fold reduction in the mean total secretion weight during the 3 days of medication when compared with placebo. The reductions were statistically significant on day 2 and 4 after virus challenge for the mean clinical score and the mean nasal secretion weight, respectively. However, following cessation of medication on day 3 after virus challenge, the mean clinical score and mean nasal secretion weight increased in the treated subjects. We are at present conducting further trials in which the drug is given over a longer period and in this paper we shall report these findings also.

R32.4

THE PROPHYLACTIC AND THERAPEUTIC EFFECT OF ZINC GLUCONATE ON RHINOVIRUS COLDS. D. A. J. Tyrrell, W. Al-Nakib, P. G. Higgins, and I. G. Barrow, MRC Common Cold Unit, Salisbury, U.K.

In double-blind placebo controlled trials in isolated volunteers of whom 29 received zinc and 28 received placebo, we have studied the prophylactic effect of zinc on colds that follow inoculation with human rhinovirus 2 (HRV-2). These studies showed that zinc gluconate lozenges (23 mg) given every two hours while awake, generally reduced the mean total clinical score when compared with placebo (5.7 vs 8.3 for the placebo). However these reductions were only statistically significant on the first day after inoculation.

When zinc was given therapeutically i.e. after symptoms had begun, it reduced the mean total clinical score from 41 in the placebo group to 27 in the zinc treated group. These reductions were statistically significant on days 4 and 5 after medication. Furthermore, medication with zinc also reduced the mean total nasal secretion weight from 51.5 in the placebo group to 22 in the zinc treated group and these reductions were statistically significant on days 2 and 6 after medication. However there was no reduction in the amount of virus shed.

It was concluded that medication with zinc had a clear effect on the signs and symptoms of the common cold caused by rhinoviruses, but the mechanism of its action remains obscure.

R32.5

GENETIC BASIS FOR RESISTANCE TO RIMANTADINE EXHIBITED BY INFLUENZA A VIRUSES ISOLATED FROM PATIENTS DURING TREATMENT OF INFLUENZA. R.Belshe, A.Hay, C.Hall, J.J. Skehel, National Institute for Medical Research, London, U.K.

Resistance to the antiviral compounds amantadine and rimantadine is readily induced in the laboratory, and resistant viruses manifest an amino acid change in the transmembrane portion of the M2 protein. We determined the sequence of the M gene of four influenza A viruses isolated from two patients either before or after treatment with rimantadine. These viruses were chosen for study because they manifested a change in susceptibility to rimantadine from sensitive prior to antiviral therapy to resistant during drug therapy. The pre-therapy isolates were identical to influenza A/Hiss/1/85 in predicted amino acid sequence of the M2 protein. Both post therapy isolates contained a single base change at position 806 resulting in a codon change for amino acid 31 of the M2 protein from AGT (serine) to AAT (asparagine). This change is identical to that observed in the transmembrane portion of human or avian influenza A viruses following tissue culture passage in the presence of amantadine. Our findings indicate that the basis of resistance to rimantadine is the same among resistant viruses cultivated in the laboratory or arising in patients during therapy.

R32.6

THE PERSISTENT ACTIVITY OF BRL 39123 AGAINST HUMAN HERPESVIRUSES. M.R. Boyd, T.H. Bacon, C. Patience, D. Sutton, R.A. Vere Hodge and M. Cole. Beecham Pharmaceuticals Research Division, Epsom, Surrey, England.

The acyclic guanine derivative BRL 39123 has potent activity in plaque reduction assays against herpes simplex virus type 1 (IC_{50} 0.3μg/ml) and type 2 (IC_{50} 1.5μg/ml) and varicella zoster virus (IC_{50} 3.1μg/ml). Good selectivity is exhibited since no morphological abnormalities are seen when human, monkey or canine cells are incubated in presence of 100μg BRL 39123/ml for up to five days. In addition, inhibition of human (MRC-5) cell replication occurs only at high concentration (IC_{30} >100μg/ml). The antiviral activity of BRL 39123 in virus yield reduction assays is much more persistent than that of acyclovir.

Like acyclovir, BRL 39123 is efficiently phosphorylated only in virus infected cells, but the triphosphate has greater stability than the triphosphate of acyclovir.

In DBA/2 mice infected intraperitoneally with HSV-1 (SC16) a single subcutaneous dose of 5.5mg BRL 39123/kg caused greater reduction in peritoneal virus replication than a 50mg/kg single subcutaneous dose of acyclovir. In the same model, a single dose of 50mg BRL 39123/kg was at least as effective as three 50mg/kg doses of acyclovir.

This potent and persistent activity suggests that BRL 39123 may be useful in the treatment of human herpesvirus infections.

R32.7

IN VITRO ACTIVITY OF R 61837, A NEW ANTIRHINOVIRUS COMPOUND
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Janssen Pharmaceutica, Beerse, Belgium.

The newly synthetized compound 3-methoxy-6-[4-(3-methylphenyl)-piperazinyl]pyridazine (R 61837) was found to be a potent and specific inhibitor of human rhinoviruses.

Different rhinovirus serotypes varied widely in susceptibility to the antiviral agent. The minimal inhibitory concentrations for 50 % CPE inhibition in Hela cells ranged from 0.002 to 32 µg/ml. Of 43 serotypes tested, 32 (74 %) were susceptible to concentrations below 10 µg/ml. On the other hand, R 61837 was well tolerated by Hela cells. The dose at which the growth of cell colonies was inhibited by 50 % after 6 days was 181 µg/ml. Other RNA or DNA viruses were not affected by the compound.

The effects of R 61837 on the yields of several representative rhinovirus serotypes were studied in single round high multiplicity infections. A concentration of 1 µg/ml reduced the yield of the most susceptible serotypes by a factor of 1000 to 10,000.

The compound inactivated some but not all susceptible serotypes. The inactivation was time and concentration dependent and could not be reversed by dilution or chloroform extraction.

Experiments using human nasal polyp cultures showed that 0.1 µg/ml of R 61837 completely inhibited the replication of rhinovirus serotype 9 in their target cells.

R32.8

MAPPING THE GENOMIC LOCATION OF THE GENE ENCODING ISATIN-β-THIOSEMICARBAZONE RESISTANCE IN VACCINIA VIRUS MUTANT BY MARKER RESCUE.
O. Gur and E. Katz, Department of Virology, Hebrew University - Hadassah Medical School, Jerusalem, Israel.

Isatin-β-thiosemicarbazone (IBT) is an efficient antiviral drug active against pox viruses. It inhibits the virus during the late stages of its growth cycle.

We wished to map the gene of the IBT-resistant mutant of vaccinia virus which confers resistance to IBT. The DNA of the IBT resistant mutant was partially cleaved with MboI and the 30 kilobase (kb) DNA fragments formed were randomly cloned into the cosmid pHC79 following its cleavage with BamHI. Clone 271, which contains the I, G, L, J and H HindIII DNA fragments of the IBT resistant mutant of vaccinia virus, rescued IBT resistant mutant from BSC1 cultures pre-infected with the wild-type strain of vaccinia virus more efficiently than all other clones examined. It is suggested that vaccinia virus gene, which is involved with the antiviral activity of IBT, is located within the third 30 kb portion of the 180 kb of vaccinia virus genome.

R32.9

N-METHYLISATIN-β 4'; 4' DIETHYLTHIOSEMICARBAZONE - A SPECIFIC INHIBITOR OF MOLONEY LEUKEMIA VIRUS PROTEINS.
D. Konen, L. Sherman and Y. Teitz
Biochemical Virology, Human Microbiology, Sackler Faculty of Medicine, Tel Aviv University.

The specific inhibition of Moloney Leukemia Virus (MLV) by N-methylisatin β 4'; 4 - diethylthiosemicarbazone (M-IBDET) was studied. At concentration of 17 µM, virus production was suppressed by 80% and the synthesis of the precursors' for 'gag' proteins - Pr-80 and Pr-65 were reduced by 70%. Northern blot analysis of RNA from M-IBDET treated cells showed that the amount and the size of 35 S and 22 S subgenomic viral RNA were not affected. Translation activity was tested in rabbit reticulocyte cell free protein synthesizing system. Translation products were immunoprecipitated with antiserum directed against the viral p-30. PAGE analysis of the immunoprecipitates revealed that poly (A) RNA preparations derived either from treated or untreated cells, had been translated equally well into the viral precursors 'gag' proteins Pr-80 and Pr-65. Translation of viral RNA was examined by addition of M-IBDET into the translation system. Total poly (A) RNA and hybrid selected specific viral RNA was tested. PAGE analysis of the immunoprecipitated translation products showed a decrease in viral precursors $Pr\text{-}80^{gag}$ and $Pr\text{-}65^{gag}$. Thus demonstrating a direct specific inhibition of translation of viral RNA by M-IBDET.

R32.10

COMPARISON OF INTERFERON ALFA-N1, PODOPHYLLIN OR COMBINATION THERAPY FOR RECURRENT GENITAL WARTS.
L. Eron, G. Delgado, J. Douglas, F. Flowers, M. Rettenmaier, F. Britt and P. Weck. Fairfax Hospital (Falls Church, VA), Georgetown Univ. Medical Center, Denver Disease Control Service, Univ. of Florida, Univ. of California Irvine, and The Burroughs Wellcome Company.

This on-going trial has randomized 189 patients with first recurrence genital warts to receive interferon alfa-n1 (WELLFERON-W), podophyllin (P), or a combination of these two treatments (W+P). Interferon (5 megaunits) is administered systemically daily, Mondays through Fridays, every other week for six weeks. Podophyllin is applied topically once weekly as long as warts are present, up to a maximum of six weeks. Patients are followed for six months for recurrence of warts. Responses in 146 patients are classified as cleared (100% clearance), improved (≥50% clearance), or no response (<50% clearance) based on lesion measurements.

	Females				Males			
Regimen	N	Clear	Impr.	NR	N	Clear	Impr.	NR
W	20	15%	55%	30%	23	9%	30%	61%
P	26	35%	46%	19%	24	21%	58%	21%
W+P	26	42%	46%	12%	27	33%	59%	8%

Results to date suggest that systemic interferon can be combined with podophyllin to increse clearance rates of recurrent genital warts and that females may respond better than males to interferon alone.

R32.11

ANTIVIRAL EFFECT OF PROGESTERONE:
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Institute for Comparative Tropical Medicine and Parasitology, Faculty of Veterinary Medicine, University of Munich, Munich, Federal Republic of Germany.

The influence of progesterone on orthopox viruses was investigated in vitro and in vivo. Orthopox virus cameli induced cytopathogenic effects in Vero cells were markedly reduced in the presence of the hormone. Dose dependent restriction of viral replication was evaluated by infectivity titration and immunological antigen determination. Progesterone concentrations of 5 µg/ml tissue culture medium depressed the production of viral particles nearly totally. The results obtained by two different methods indicate that probably later stages of viral synthesis are affected when lower dosages of progesterone are used.

The antiviral activity of progesterone in vivo was confirmed by using rabbits for intradermel vaccinia virus infections.

R32.12

INTRANASAL INTERFERON-ALFA-2B TREATMENT OF NATURAL RHINOVIRUS COLDS.
FG Hayden, DL Kaiser, and JK Albrecht. Departments of Internal Medicine and Pathology, University of Virginia, Charlottesville, VA and Schering Corporation, Kenilworth, NJ U.S.A.

Intranasal interferon-alfa-2b(IFN) has proven efficacy in preventing natural rhinovirus (RV) colds. The current study assessed the therapeutic activity of intranasal IFN in natural colds. Under double-blind conditions, patients symptomatic ≤48 hours were randomly assigned to IFN 20 MU/d, IFN 10 MU/d, or placebo(P) given by intranasal spray 4 times/day for 5 days.

The IFN-10(n=74), IFN-20(n=74), and P(n=72) groups were comparable in proportions of laboratory-documented RV colds (51, 66, and 61%, resp.) and mean duration of pretreatment symptoms (27, 27, and 26 hrs, resp.). The median duration of all colds (subjective assessment) tended to be longer in the IFN-20(10d) than the IFN-10(8d) or P(8d) groups (p=0.06, vs IFN-20). No differences in respiratory symptom scores or resolution of specific symptoms were found. In those with proven RV colds treated within 24 hrs, the duration also tended to be longer in the IFN-20(9d) than IFN-10(6d) or P(6d) groups (p=0.06). Nasal washings on treatment day 5 yielded RV more often in P(7/13) than IFN-10 and IFN-20(5/30) patients (p=0.04). Blood in nasal mucus was reported by significantly more IFN-10(16%) or IFN-20(18%) than P(4%) subjects during treatment. Therapy for presumed secondary bacterial infections was given in 8 IFN-20, 4 IFN-10 but no P recipients (p<0.01, vs IFN-20). IFN nasal spray was not effective treatment for natural colds and was associated with discernible toxicity.

R32.13

USE OF ESCAPE MUTANTS TO MAP SITES CRITICAL FOR ACTIVITY OF WIN DRUGS. B. Heinz, R. Rueckert. Biophysics Laboratory, University of Wisconsin, Madison, WI, USA.

WIN 51711 and 52084 are experimental drugs that are effective against picornaviruses. These drugs bind to a well-defined site within the protein capsid and block viral uncoating. We have detected two classes of human rhinovirus 14 (HRV14) mutants which are resistant to WIN 52084: a low resistance class (L) which plaque at 2X minimum inhibitory concentration (MIC), with a frequency of 10^{-3}-10^{-4} mutants per wild type plaque; and a high resistance class (H) which plaque at 40X MIC, with a frequency of 10^{-5} mutants per wild type plaque. Mutants dependent on the drug for efficient replication (D-mutants) have also been observed.

To identify amino acids important for drug activity we are characterizing drug-resistant mutants by sequencing regions of the RNA genome encoding the drug binding site. To date, we have mapped H-mutants to two residues in the drug-binding pocket: Valine #188 (at the hydrophobic end of the pocket) has changed to residues with longer side chains (methionine or leucine); and Cysteine #199 (near the "ion channel" leading from the floor of the capsid to the RNA) has changed to tryptophan. Additional residues are also involved, since some mutants do not map to either of these sites. These results show that escape mutant analysis, which we used to identify antibody binding sites on HRV14, is also applicable to studying drug binding.

R32.14

IN VITRO AND IN VIVO PHLEBOVIRUS-INHIBITORY ACTIVITY OF RIBAVIRIN. R.W. Sidwell, J.H. Huffman, B.B. Barnett, and D.Y. Pifat. Dept. of Animal, Dairy and Veterinary Sciences, Utah State Univ., Logan, Utah, and Dept. of Antiviral Studies, U.S. Army Medical Research Institute for Infectious Diseases, Ft. Detrick, Frederick, Maryland, USA.

Ribavirin (1-ß-D-ribofuranosyl-1,2,4-triazole-3-carboxamide) was markedly inhibitory to Adames and Balliet strains of Punta Toro virus (PTV), a Phlebovirus, in LLC-MK_2 cells, using inhibition of cytopathic effect and reduction of intra- and extra-cellular virus titer. The 50% effective dose was 1-10 µg/ml, and the Virus Rating was 1.3-1.5. Marked activity was also seen against Adames strain PTV infections induced by subcutaneous (s.c.) inoculation of 3-4 week old C57BL/6 mice when ribavirin was administered s.c. twice daily for 5 to 7 days beginning 4 hr pre-virus inoculation. Activity was expressed as increased survivors, reduced hepatic icterus, elimination of positive glutamic oxalic and pyruvic transaminase levels in the serum, and inhibition of recoverable virus from sera and livers of infected mice. The maximum tolerated dose of ribavirin in these animals was 75 mg/kg/day, and the minimum effective dose was 4.7 mg/kg/day. Inoculum size of PTV affected ribavirin's efficacy; when the drug was evaluated against 0.1, 1, 10 and 100 LD_{50} of PTV, activity was seen at all levels, but a ribavirin dose of 9.4 mg/kg/day was most effective at the 0.1 and 1 LD_{50} virus levels. Ribavirin treatment of the intracerebrally inoculated Balliet strain PTV was not effective.

R32.15

ACYCLOVIR 5% MODIFIED AQUEOUS CREAM AND HERPES LABIALIS: REPORT OF A CLINICAL TRIAL. G.W. Raborn, W.T. McGaw, M. Grace, L.D. Tyrrell, S. Samuels. Department of Stomatology, Faculties of Dentistry and Medicine, University of Alberta, Edmonton, Canada.

Eighty patients previously culture confirmed positive for herpes labialis were entered into a randomized, placebo controlled, double blind study to test the effects of 5% ACV in a modified aqueous cream base. Patients applied the study medication every 4 hours for five days beginning at earliest sign of prodrome. No virological data was collected during the treated episodes. Forty-eight patients were followed through 2 episodes each on either placebo or ACV cream and the data was analyzed. ACV treated patients showed a trend toward faster healing during both episodes followed but the results were not significant statistically. ACV treated patients also tended to have their lesions crust first and experienced a loss of crust earlier than the placebo treated patients although not significantly.

R32.16

COMPARATIVE STUDY ON THE MECHANISM OF ACTION OF BVaraU AND IVDU AGAINST VZV REPLICATION. T. Yokota, K. Konno, S. Mori, S. Shigeta, H. Machida[1], E. De Clercq[2]. Dept. of Bacteriology, Fukushima Medical College, Fukushima, Res. Lab., Yamasa Shoyu Co. Ltd[1]., Choshi, Japan. Rega Institute, Katholieke Universiteit Leuven[2], Leuven, Belgium.

To investigate the mechanism of action of (E)-5-(2-bromovinyl)-1-ß-D-arabinofuranosyluracil(BVaraU) and (E)-5-(2-iodovinyl)-2'-deoxyuridine(IVDU) for varicella-zoster virus (VZV) replication, we compared the incorporation of two drugs into VZV DNA in virus infected cells using both [^{14}C]-labelled BVaraU and [^{125}I]-labelled IVDU.

BVaraU and IVDU are very potent and selective antiherpes agents. The basis for the selective action of BVaraU and IVDU against VZV replication resides in their enhanced affinity for the virus-encoded thymidine kinase(TK). The triphosphate derivatives of BVaraU and IVDU are competitive inhibitors of VZV DNA polymerase. IVDU is incorporated into VZV DNA in VZV infected cells, and the degree of inhibition correlates well with the amount of drugs substituted for dThd in viral DNA. In marked contrast, the detectable incorporation of BVaraU into VZV DNA is not proved in VZV infected cells, although BVaraU is taken up and phosphorylated by cells infected with VZV. BVaraU may not serve as alternate substrate for VZV DNA synthesis, or BVaraU may be incorporated into VZV DNA and may suppress further DNA elongation.

R32.17

NUCLEOSIDE TRANSPORT IN CELLS INFECTED WITH HERPES SIMPLEX VIRUS. G. Palù, S. Stefanelli, P. Palatini, R.E. Handschumacher. Institute of Microbiology and Department of Pharmacology, Faculty of Medicine, Universities of Padova (Italy) and Yale (U.S.A.).

Experiments on the transport of thymidine (dThd) by cells infected with herpes simplex virus type 1 (HSV-1) were carried out to elucidate the controversial aspect of "membrane leakiness" after viral infection. Initial rates of ^{3}H-dThd influx were determined in HeLa-S3 cells at different time intervals post infection (p.i.) with a wild type TK^+ strain and a TK^- variant. Uptake of dThd was progressively increasing from the beginning of infection up to 8 hr p.i. but declined below control values at 24 hr p.i. Meanwhile the number of transporters gradually diminished. Kinetic parameters were also changed, since dThd uptake ceased to be a saturable phenomenon. These results revealed that passive diffusion of dThd was contributing to some extent to the overall rate of nucleoside influx soon after infection.

On the other hand, an "impermeant" inhibitor of protein synthesis such as hygromycin B became active only when protein synthesis was already depressed in infected cells. These observations imply that an effective chemotherapy with selective antiherpes nucleoside compounds may also depend on the degree of affinity of these drugs for the cell transporters.

R32.18

ATTACHMENT OF HERPESVIRIONS & FUSION REQUIRE CARBOHYDRATES. H.A. Blough, C. Baron, M.J. Massare. Department of Ophthalmology, School of Medicine, University of Pennsylvania, Philadelphia, U.S.A.

Although the entry of herpesvirus (HSV-1) requires carbohydrates, their role is unclear. Peptidyloligosaccharides were isolated from cell surfaces of BHK-21 or green monkey kidney cells & hydrolyzed sequentially with pronase & carboxypeptidases; alternatively, hydrazinolysis was used & the oligosaccharides (OG) purified by lectin affinity & molecular sieve column chromatography. An exogenous fusion factor (FF), obtained from the medium of HSV-infected cells, was purified on discontinuous albumin (12-40%, v/v) followed by metrazamide gradients (2.7-30%, v/v). The ability of the OG fractions and/or simple monosaccharides to block attachment or fusion was ascertained by competition studies as well as measuring the binding of [^{3}H]-OG (endogenously labeled) to HSV. OG (2 ug) could block the attachment of highly purified [^{3}H]-HSV by 1-2 $\log_{10}$; whereas it had no effect on fusion. Conversely, pretreatment of cells with fucose (10^{-5} M), and to a lesser extent Gal inhibited fusion, but not attachment. Analysis of FF by t.l.c. & g.l.c. showed an enrichment of fucosylated glycerol ethers in FF vs uninfected plasma membranes. The OG, responsible for HSV attachment were N-linked & complex in nature; removal of NeuNAc or SO_4 had no effect. In conclusion, host induced modifications (glycosylation) play an important role in HSV entry & the receptors for attachment & fusion are different.

R32.19

MISMATCHED DOUBLE-STRANDED RNA (AMPLIGEN) PROTECTS TARGET CELLS FROM HIV INFECTION AND REDUCES THE CONCENTRATION OF 3'-AZIDO-3'DEOXYTHYMIDINE (AZT) REQUIRED FOR VIRUSTATIC ACTIVITY. William M. Mitchell, David C. Montefiori, W. Edward Robinson, and William A. Carter, Vanderbilt University, Nashville, Tennessee, USA and Hahnemann School of Medicine, Philadelphia, Pennsylvania, USA.

The biological response modifier $rI_n \cdot r(C_{12} \cdot U)_n$, generally referred to as mismatched double-stranded (ds) RNA or Ampligen® was able to protect target lymphoblastoid cells in vitro from infection by the human immunodeficiency virus (HIV). Significant protection of the highly HIV-permissive T-cell line C3 is observed with Ampligen in the 10-50 µg/ml concentration range. Similar results are observed at 50 µg/ml in CEM cells. When administered simultaneously with sub-virustatic concentrations of azidothymidine (AZT), protection of target cells from HIV infection is increased. At higher doses of AZT tested, the virustatic activity observed appeared to be in a synergistic virustatic relationship with Ampligen. Moreover, in combination with Ampligen at least a five-fold reduction in AZT concentration can be used in order to obtain equivalent virustatic activities. Thus, combined therapy with Ampligen and AZT can be expected to be more beneficial to ARC or AIDS patients since current AZT regimens of apparent clinical effectiveness are associated with significant toxicities which undermine its therapeutic potential.

R32.20

CLINICAL THERAPEUTIC EFFICACY OF INTRAVENOUS RIBAVIRIN TREATMENT OF HEMORRHAGIC FEVER WITH RENAL SYNDROME (HFRS): RANDOMIZED, DOUBLE-BLIND, PLACEBO CONTROLLED TRIAL IN THE PEOPLE'S REPUBLIC OF CHINA. J. W. Huggins[1], C. M. Hsiang[4], T. M. Cosgriff[2], M. Y. Guang[4], J. J. Smith[2], Z. A. Wu[4], J. W. Le Duc[3], Z. M. Zheng[4], J. M. Meegan[2], C. N. Wang[4], X. E. Gui[4], K. W. Yuan[4], T. M. Zhang[4]. [1]Department of Antiviral Studies, Virology Division; [2]Medical Division; [3]Department of Epidemiology, Disease Assessment Division, U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Maryland, 21701-5011, USA. [4]Hubei Medical College, Wuhan, People's Republic of China.

HFRS is a serious viral disease with renal and hemorrhagic components, which results in significant mortality. The antiviral drug ribavirin has shown activity against Hantaan virus, the cause of HFRS, in vitro and in a suckling mouse model. To evaluate efficacy in humans, over 190 serologically confirmed (by IgM ELISA) cases, classified as grave (4%), severe (21%), moderate (53%) and mild (22%) by the Chinese classification system were enrolled into 9 collaborating hospitals in an endemic area of Hubei Province, PRC. Patients were randomized between intravenous ribavirin and placebo treatment which was initiated within six days of onset of fever using a double blind study design. Drug efficacy will be presented as assessed by mortality, clinical parameters, serum and urine chemistry tests, including effects on electrolytes, renal and liver function tests, hematology and coagulation profiles.

R32.21

ORAL EFFICACY OF AN ACRIDINE DERIVATIVE (AD) IMMUNOMODULATOR AGAINST RIFT VALLEY FEVER VIRAL (RVFV) INFECTION IN MICE. M. Kende, W. Rill, J. Smith, M. Derevjanik and P.G. Canonico. U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland, U.S.A.

AD (CL246,738, Lederle) which is undergoing Phase I clinical trials in patients with cancer and in immunocompromised hosts was evaluated for its efficacy against lethal RVFV infection alone or in combination with ribavirin. When administered simultaneously or 1 or 2 days post-challenge, a single dose of 50 mg/kg AD yielded 60% survivors. For maximum prophylactic efficacy (63% survivors) multiple doses were required, but only 3 or 4 doses were needed therapeutically to elicit 100% protection. Multiple doses were well-tolerated without changes in liver and heart muscle serum enzyme levels. In infected, untreated mice, the functional enzyme levels were elevated; however, the values were normal during and after therapy. Ex-vivo, therapeutic dose(s) of AD modulated liver and spleen NK cytotoxic reactivity against YAC target cells and stimulated the serum interferon level (~10000U/ml), without substantial hyporesponsiveness. When lower doses of AD and ribavirin were administered separately 1-day postinfection, the compounds had no therapeutic efficacy; however, with combined therapy, there were 80% survivors. This and other studies indicate the potential of this immunomodulator for human use against viral infections alone or in combination with ribavirin.

R32.22

PROTEASE INHIBITORS SUPPRESS THE IN VITRO AND IN VIVO REPLICATION OF ROTAVIRUSES. Steven L. Vonderfecht, S.B. Wee, and Robert Yolken, Johns Hopkins School of Medicine, Baltimore, Md.

Rotavirus replication is enhanced by intestinal proteases which serve to cleave surface viral proteins. We thus investigated the ability of protease inhibitors to suppress the in vitro and in vivo replication of rotaviruses. A wide variety of protease inhibitors were able to prevent the growth of rotavirus strains in cell culture as well as to suppress intestinal viral replication and restrict disease transmission in a mouse model of rotaviral infection.

Effective inhibitors of rotaviral replication included naturally occurring glycoproteins such as ovoinhibitors and α-1-antitrypsin as well as synthetic drugs such as leuptin, bis (5-amidino-2-benzimidazolyl) methane and pentamidine. The latter compound is of interest since it is often administered to humans for the suppression of parasitic infections and is thus an appropriate drug for human experimentation. Protease inhibitors have potential for the prevention or treatment of enteric viral infections in humans and animals.

R32.23

INHIBITION STUDIES WITH FOSCARNET AND AZIDOTHYMIDINE IN HIV-INFECTED H9-CELLS.

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All retroviruses contain a unique enzyme, reverse transcriptase (RT), which is essential for the replication. Since no corresponding enzyme exists in normal mammalian cells, RT is an attractive target for attempts at inhibition of virus growth. Several inhibitors have been described and some have also been investigated in HIV-infected patients. We have studied the effects of foscarnet, amantadin, and the two isomers of azidothymidine in HIV-infected H9-cells during incubation for one month Prior to infection, $2x10^6$ cells were preincubated for 24 h with a compound. The progress of infection was monitiored by appearance of CPE, immunofluorescence and RT activity. Amantadin(100uM) and threo-AZT(1uM) had no antiviral effect, erythro-AZT(0.1uM) and foscarnet(100 uM) showed a protective effect up to 14 days and 250 uM foscarnet up to 21 days. Erythro-AZT(1uM) and foscarnet (500uM) completely controlled the infection during 28 days. After removal of the drugs, a production of new HIV occurred within 5 days.Reinstitution of the treatment had no effect on these cells. No occurrence of drug resistence of RT was observed during the experiments.

R32.24

SENSITIVITY MONITORING OF ISOLATES OF HERPES SIMPLEX VIRUS FROM PATIENTS ENROLLED IN A U.K./EUROPEAN CLINICAL TRIAL OF LONG TERM PROPHYLACTIC ORAL ACYCLOVIR (ZOVIRAX™). P.Collins, Department of Biochemical Virology, The Wellcome Research Laboratories, Beckenham, U.K.

Otherwise healthy patients with frequently recurring herpes simplex virus (HSV) infections were enrolled into an open multicentre prophylactic study of oral acyclovir. A pre-treatment virus isolate was taken for acyclovir sensitivity testing from each patient prior to entry into the trial. The patients were then protocolled to receive 800mg acyclovir in 2 or 4 divided daily doses for a period of 52 weeks. Further virus isolates were taken at the first recurrence after the cessation of therapy and from any breakthrough during therapy.

The sensitivity of 145 pre-treatment isolates from 107 patients, 61 post-treatment isolates from 52 patients and 8 breakthrough isolates from 7 patients was determined by plaque-reduction assays in Vero cells. A mean 50% inhibitory concentration (IC_{50}) for acyclovir of 0.94µM (Range 0.2-2.8µM) was obtained for the pre-treatment isolates as compared to a mean of 0.87µM (Range 0.1-2.3µM) for post-treatment isolates. There was no reduction in sensitivity observed in paired pre- and post-treatment isolates from 42 patients. Similarly no significant reductions in sensitivity were observed in the breakthrough isolates with IC_{50} values ranging from 0.42-2.8µM.

R32.25

DEPRESSION OF THE CYTOTOXIC T CELL RESPONSE TO INFLUENZA VIRUS INFECTION IN MICE BY RIMANTADINE. J.E. Herrmann, M. Bruns, K. West, F.A. Ennis. Division of Infectious Diseases University of Massachusetts Medical School,Worcester, MA, U.S.A.

We have evaluated, in a murine model, the use of rimantadine for the treatment of influenza virus infection. Evaluation included determination of antiviral activity, measured by suppression of virus concentration in the lungs of treated mice, and the effect of rimantadine on the cellular immune response to virus infection. This was assessed by analysis of the influenza virus specific MHC restricted cytotoxic T lymphocyte (CTL) response. Two routes of rimantadine administration were tested for treatment of mice infected with influenza A/Port Chalmers (H3N2) virus, intraperitoneal and oral. It was found that oral administration via drinking water was the most effective, and resulted in up to a 4 $\log_{10}$ reduction in virus titer at doses (in mg/kg) equivalent to that recommended for human infections. At these doses, it was also found that by day 12 post infection there was a 50 to 60% reduction ($p<0.02$), at effector: target cell ratios of 60 and 30:1, of the virus specific CTL response. This reduction occurred when rimantadine was given prophylactically plus therapeutically, or as a therapeutic agent only. The results demonstrate that in addition to antiviral effects, rimantadine treatment results in depression of the CTL response to influenza virus.

R32.26

IMMUNOSUPPRESSION BY 9-[2-HYDROXY-1-(HDROXYMETHYL)ETHOXYMETHYL]GUANINE (DHPG) IN NORMAL SUBJECTS AND IN PATIENTS FOLLOWING MARROW TRANSPLANT (MTx). R.A. Bowden*, J. Digel, E.C. Reed, J.D. Meyers, Fred Hutchinson Cancer Research Center, Seattle, WA, USA

DHPG, a nucleoside analogue of acyclovir with improved in vivo and in vitro activity against cytomegalovirus (CMV), interferes with cellular DNA synthesis and therefore may affect cell-mediated immunity which is potentially important to the outcome of CMV infection. Lymphocyte transformation response (LTR) to PHA and CMV antigen using peripheral blood lymphocytes (PBL) from 7 seropositive normals was evaluted in the presence of DHPG (1-50 ug/ml). LTR to both CMV and PHA decreased a mean of 17%, 41% and 54% at 1, 5 and 10ug/ml of DHPG, respectively. There was no effect on IL-2 production or on cytotoxic responses to CMV-infected and uninfected targets at similar concentrations of DHPG. LTR were studied in 8 MTx patients receiving DHPG at 7.5 mg/kg/day. Mean peak DHPG levels were 3.6 ug/ml and troughs 0.3 ug/ml. 6 patients had increases in LTR to PHA on DHPG. Only 1 had a decreased response. In contrast, only 1/7 patients whose cells had negative LTR to CMV before treatment became positive on DHPG. The one patient with a positive LTR prior to DHPG had a negative response on DHPG which became positive again after DHPG. In summary, both in vitro and in vivo studies suggest that DHPG may result in substantial immunosuppression, possibly interfering with successful anti-CMV treatment.

R32.27

PHARMACOKINETICS AND CLINICAL TOLERANCE OF AEROSOL RIBAVIRIN IN THE HIGH RISK ELDERLY. J.M. Bernstein[1,2], H. Liss[1,2] and J.D. Connor[3], Dept. Med., Wright St. U. Sch. Med.[1] and VAMC Dayton, OH[2] and Dept. Ped., UC San Diego Sch. Med.[3], USA.

Ribavirin (R) is an anti-viral agent that has been found to be effective in the aerosol treatment of influenzal and respiratory syncytial virus infections. There are little available data regarding the use of aerosol R (aR) in elderly persons with chronic heart or lung disease.

We evaluated the pharmacokinetics and clinical tolerance of high dose aR in persons at high risk for complications of influenza. 7 men and 1 woman (mean age 63.5 years) were studied; 7/8 had moderate to severe obstructive airways disease. The study groups received aR at either 40 mg/ml in distilled H_2O (dH_2O) x 6h, repeated every 12h (Group A) or 60 mg/ml in dH_2O x 2h, every 8h (Group B); both regimens were continued for 96h. All patients continued their standard medications (eg: bronchodilators). All patients were tolerant of their treatment regimen. No deterioration in pulmonary function was demonstrable related to therapy nor were any abnormalities in either hematologic or blood chemical parameters found up to 28d post therapy. Group A had a rapid rise in mean serum R to 8.26 µM at the end of day 1; this rose to 10.1 µM after day 4. Group B reached a maximum serum R of 4.97 µM at the end of day 1 with a peak of 11.5 µM at the end of day 4. We conclude that high dose aR is well tolerated by elderly, high risk patients. Further trials of this agent by the pulmonary route should be undertaken.

R32.28

COMBINE ANTIVIRAL EFFECT OF RIBONUCLEASE AND REMANTADIN ON THE REPRODUCTION OF INFLUENZA A VIRUSES.,A.Uzunova. Institute of microbiology, Bulgarian Academy of Science,Sofia,Bulgaria

A combine antiviral effect of ribonuclease and remantadin on the reproduction of 3 types of influenza viruses with different antigenicall characteristics have been establish.

The results were presenting on the basis of decrease of haemagglutining and infectious activity of treated viruses in comparison with untreated and on the basis of haemadsorbtion and plaqueforming test.

We have come to nothing in ouer experiments to obtain a resistent variants of influenza viruses in chick embryons and in the some cultures using combine effective antiviral concentrations of ribonuclease and remantadin.

R32.29

A MODE OF ACTION OF ANTIVIRAL SUBSTANCE, DIOXOHEXAHYDROTRIAZINE AND ITS SELECTIVE MECHANISM BETWEEN POTATO VIRUS X AND TOBACCO MOSAIC VIRUS. K. Ko, T. Shimizu, K. Yoneyama, I. Yamaguchi, *K. Showhara and *N. Oshima.
The Institute of Physical and Chemical Research, Wako, Saitama, Japan. *Japan Plant Protection Association, Ushiku, Ibaraki, Japan.

Since G. Schuster reported on dioxohexahydrotriazine (DHT) as an antiviral substance in 1978, its effect on plant virus diseases has been discussed. As previously reported, the authors found that DHT has a broad effective range on plant virus disease protection by changing its application method and formulation. There are, however, some remarkable specificity observed on their disease developments.

This report is concerned with its mode of action and selective mechanism of DHT between PVX and TMV. The tested DHT and ^{14}C-(U)DHT were 99% pure and used on tomato seedlings (four-weeks growth) infected with PVX and TMV. The behaviors of viruses and chemical on the host plants indicated that DHT inhibits translocation of mainly PVX from infected cells (or tissues) to the healthy cells, whereas similar effect on TMV was not so remarkable. The result of an experiment done to confirm the above data showed that the amount of DHT taken in PVX-RNA fraction is larger than that taken in TMV-RNA fraction seems to be attributed to the difference in sensitivity of the two viruses against DHT.

R32.30

EFFECT OF AN URIDINE DERIVATIVE ON HERPES SIMPLEX KERATITIS IN RABBITS AND VAGINAL INFECTION IN GUINEA PIGS; P. Vilas, C.Pérez,S.Pérez, D.G. Villaldn, A. Garcia Ganzedo, C, Gil Fernandez and M.T. Garcia López. Centro de Investigaciones Biológicas and Instituto de Química Médica. Madrid, SPAIN.

An uridine-5-diphosphate glucose derivative active in vitro against herpes simplex type 1 and 2 viruses, was assayed on virus infected rabbits corneas. The infected eyes were treated by drug instillation thrice daily and evaluation of ocular lesion was performed slit-lamp biomicroscopy. The compound 5'-O-[[[(2*,3*,4*,6*-tetra-O-benzoil-α-D-glucopyranosyl) oxi] carbonil] amino] sulphonil] uridine, shows a moderate antiviral activity, resulting in a reduction in the severity of clinical illness during acute infection.

Vaginal infection of guinea pigs with herpes simplex type 2 virus was treated topically by instillation twice daily with the compound. The effect on clinical evolution was related with viral shedding from the genital tract, and a moderate reduction of both parameters respect to the infected untreated controls was observed.

R32.31

ANTIVIRAL ACTIVITY OF URIDINE -5' DIPHOSPHATE ANALOGUES AGAINST SEVERAL VIRUSES IN VERO CELLS. C. Gil Fernandez, C. Péres, S. Péres, P. Vilas, F.G. de las Heras, A. Garcia Ganzedo. Centro de Investigaciones Biológicas and Instituto de Química Médica, Madrid, SPAIN.

Twenty four analogues of uridine -5' diphosphate glucose were screened against herpes simplex type 2, vaccinia, Sindbis and African swine fever viruses. After the screening, the compound 5'-O-[[[[(2',3',4',6'-tetra-O-benzoil-α-D-glucopyranosyl) oxi] carbonil] amino] sulfonil] uridine, the synthesis of which has been reported (1), was selected for an extensive study.

This compound shows antiviral activity in vitro against all the virus tested. The replication of herpes virus type 2 and African swine fever virus is completely inhibited at 100 μg/ml and 150 μg/ml respectively, in less extent vaccinia and Sindbis viruses are also inhibited.

The compound inhibits an early and late step of viral replication, this indicates that the mechanism of action may be complex and may interfere with two or more steps of viral replication.

(1) M.J. Camarasa et al. J.Med.Chem. 28, 40-46 (1985)

R32.32

SYNTHESIS AND ANTIVIRAL ACTIVITY OF 5'-O-(N'-SUBSTITUTED SULFAMOIL) URIDINES. S.Péres[1] J.Plandor,[2] C. Péres,[1] M.T. Garcia López,[2] A. Garcia Ganzedo,[1] F.G.de las Heras,[2] C. Gil Fernandez,[1] P. Vilas[1], P.P. Mendez Castrillón,[2] Centro de Investigaciones Biológicas(1) and Instituto de Química Médica (2). Madrid. SPAIN.

These compounds have been prepared by reaction of alcohols with chlorosulphenyl isocyanate and isopropylidene uridine, and by reaction of alkylsulfamil chlorides with isopropylidene uridine. They have been tested in vitro for their antiviral activity against herpes simplex type 2 (HSV-2), vaccinia (VV), Sindbis and African swine fever (ASFV) viruses.

Cytotoxicity (MTC_{50}) and reduction of virus yield (MIC_{50}) have been determined and the therapeutic index was calculated.

Some of this compounds have medium therapeutic index (10 to 20) against HSV-2 and VV viruses. One of them have a high therapeutic index of 440 against ASFV.

Out of this compounds only two have shown virucidal effects.

This results indicate a promising antiviral activity and provide the basis for further investigation of new derivatives with higher selective potencial.

R32.33

PROTECTIVE EFFECT OF ANTIVIRAL DRUGS AND IMMUNOMODULATORS UPON INFLUENZA. M.Indulen, G.Ryazantseva, D.Dzeguze, R.Feldblum, V.Bubovich. A.Kirchenstein Institute of Microbiology, Latvian SSR Acad.Sci.

The investigation was aimed at the search for combinations of drugs with various modes of action, effective suppressors of influenza virus reproduction in cell culture, preventing animals from lethal influenza infection. The investigations were carried out with influenza viruses: FPV/Weybridge (H7N7) in CEF culture, A/Bangkok/1/79, A/Aichi/2/68 (H3N2) in MDCK culture, A/Aichi/2/68 - on white mice. We studied the antiviral effect of rimantadine, adapromine, ribamidyl, dipyridamole and immunomodulators - inosiplex, tuftsin, quercetin and their combinations. Immunomodulators increased the viability of animals more than 2.5 times as against the control, and intensified the protective effect of rimantadine. A combination of rimantadine and inosiplex possessed a more marked effect. A combination of two antiviral drugs with various modes of action (rimantadine+ribamidyl) or of an antiviral drug and immunomodulator (rimantadine+inosiplex or tuftsin) improved the protection of animals from influenza infection. The increase of antiviral protection in some cases was accompanied by interferon production in animal blood sera.

R32.34

DNA AND RNA VIRUSES ARE INHIBITED BY XANTHATE COMPOUNDS. G. Sauer, E. Amtmann, K. Müller-Decker. Institute for Virus Research, German Cancer Research Center, Im Neuenheimer Feld 280, 6900 Heidelberg, FRG.

Appropriately substituted xanthates display a broad-range antiviral activity which to our knowledge is unprecedented. The replication of various DNA and RNA viruses is inhibited by tricyclodecan-9-yl-xanthate (D609) in vitro: herpes simplex, simian virus 40, bovine papilloma, vesicular stomatitis, coxsackie B4, moloney murine sarkoma virus and HIV. This effect was noticed at concentrations that left the mitotic activity of uninfected control cells unimpaired. Fully developed herpetic lesions could be cured after topical treatment of guinea pig skin. The acute toxicity i.v. ranges between 1 and 5 g/kg mouse. Xanthates do not induce interferon and they do not serve as precursors of viral macromolecules. The activity of protein kinase C appears to be the main target of inhibition, thus, cellular regulatory processes are affected by the xanthates which may be more crucial for viral growth than for the host cell.

R32.35

EFFICACY OF 1-β-D-ARABINOFURANOSYL-E-5-(2-BROMOVINYL)URACIL (BV-araU) AGAINST EXPERIMENTAL ENCEPHALITIS INDUCED BY TWO DIFFERENT STRAINS OF HSV-1 IN MICE. H. Machida. Research Laboratories, Yamasa Shoyu, Co., Ltd., Choshi, Japan.

There are some discrepancies in the observations by different researchers on the *in vivo* efficacy of anti-herpes drugs, probably because different virus strains were used as challenge viruses. The virulence of the virus used may affect the potency of test drug. Thus, therapeutic efficacy of BV-araU was tested against encephalitis in mice induced by two strains of HSV-1, whose virulence is different.

Groups of about 20 four-week-old male albino Swiss mice were infected either i.c. with 6 LD_{50} or i.p. with 30 LD_{50} of HSV-1 strain VR-3 (high virulence) or strain WT-51 (low virulence). The infected mice were treated i.p. or orally with drug twice daily for 7 days. Survival rate and time in the treated groups were compared with those in control.

Treatment with 12.5 mg/Kg of BV-araU or more for a week caused significant increase in survival rate in mice infected i.c. with strain WT-51 and most of the mice treated with 200 mg/Kg of BV-araU survived, while dosage with 200 mg/Kg was necessary for reducing mortality in strain VR-3-infected mice. Oral treatments with BV-araU were as effective as or more effective than i.p. treatments in both infected-systems. Acyclovir was much less efficacious than BV-araU in the models in which virus was inoculated i.c., while the former demonstrated much higher efficacy against i.p. infection with HSV-1 strain VR-3 than the latter.

R32.36

ACTIVITIES OF SYNTHETIC AND NATURAL SUBSTANCES ON A HEAT RESISTANT STRAIN OF TOBACCO MOSAIC VIRUS. J.K.Bakarosa, M.T.Sayed, M.M.El-Shamy. Department of Botany, Faculty of Science, University of Cairo, Giza, Egypt.

A trial has been conducted to inactivate a heat resistant strain of TMV isolated from naturally infected tomatoes by treatment with the following 8 substances:

2-oxo-3-oximino-4phenylimino pentane (1);
2-oxo-3-oximino-4(p-aminophenyl) imino pentane (2);
3-p-methoxyphenyl-3-oxobutanoic acid (3); Stomp;
Emide 50w; Sencor; fresh & powdered milk and human urine.

The effect of the said substances on the infectivity of virus was studied both in vitro and in vivo. Datura metel, a susceptible local lesion host to the virus was used. After treatment with various concentrations of the substances for different storage periods, the number of local lesions produced on at least 5 leaves from 3 plants was taken as an expression of virus infectivity and was converted to percentage of the control. Nearly complete or complete inhibition of virus infectivity in vitro was achieved when the virus was incubated for 15 min with 1000 to 10000 ppm (1),(2),(3), respectively, and undiluted natural and powdered milk. Undiluted fresh milk and 1000 ppm powdered milk caused complete or nearly complete inhibition of virus infectivity in vivo 1 h before or after inoculation with virus.

R32.37

POTENTIAL ANTIVIRAL ACTIVITY OF TUFTSIN.
M. Łuczak, H. Kleklik, V.A. Najjar*. Department of Virology, Medical Academy, Warsaw, Poland; *Protein Chemistry, Tufts University, Boston, USA.

The natural tetrapeptide tuftsin is an activator of all known functions of phagocytic cells.

In the present experiments the influence of tuftsin and its elongated analogues on the mortality of EMC virus infected NMRI mice was studied. It seems that amino acids elongation in the parent peptide chain does not result in significant increase of anti EMC virus activity.

In the next experiments a significant decrease in mortality was observed when 25ug of the tetrapeptide was given 5 days before infection with Friend Leukemia Virus. The same effect was observed when tuftsin was given 5 days before and twice a week for 3 weeks after FLV infection. Other experiments showed that development of EMC-V virus induced tumors was significantly diminished after a single injection of tuftsin or one of its analogues.

Tuftsin induced tumor necrosis activity was found in mice serum several days after ip injection of the peptide. The same effect was observed after stimulation promyelocytic leukemia cells /HL-60/ with different concentration of tuftsin.

R32.38

IN VITRO ANTIVIRAL ASSAY SYSTEM AGAINST HEPATITIS B VIRUS.
S. Suzuki[1]), M. Saneyoshi[2]), and D.L.J. Tyrrell[1])
Departments of Biochemistry and Medical Microbiology and Infectious Diseases, Faculty of Medicine, University of Alberta, Edmonton, Canada[1]), and Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo, Japan[2]).

Hepatitis B virus is one of the important targets for antiviral chemotherapy. However, it is difficult to find a selective inhibitor for hepadnaviruses since there is no in vitro assay system. We attempted to establish the in vitro assay system using the duck hepatitis B virus (DHBV) system. Culture of duck hepatocytes and in vitro experimental infection with DHBV were reported by Tuttleman, et al (J. Virol. 1986, 58:17). This system was adapted for an antiviral assay. Perfused hepatocytes from virus infected ducks were cultured and the candidate antiviral compounds were added. After the cells were harvested, DNA was extracted, electrophoresed and blotted onto nitrocellulose filter. A ^{32}P-labelled probe (DNA or RNA) was used for hybridization. 2', 3'-Dideoxy-3'-azido-thymidine, a known inhibitor of HIV infection, and other pyrimidine analogues which possess modified sugar moiety were screened for antiviral activity against DHBV using this system.

R32.39

INFLUENCE OF DIFLUOROMETHOXY SUBSTITUTION AT C-4 OF 5-SUBSTITUTED URACIL NUCLEOSIDES ON ANTIHERPES ACTIVITY. J. Reefschläger[1], C.-D. Pein[2], D. Cech[2] and D. H. Krüger[1].
Institute of Virology[1] and Dept. of Chemistry[2], Humboldt University, Berlin 1040, G.D.R.

Various 4-O-difluoromethyl (CF_2) analogues of 5-substituted (5-I-) 2'-deoxyuridine (dUrd) and arabinofuranosyluracil (araU) nucleosides were evaluated for their activity against herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) as well as against varicella-zoster virus(VZV) and human cytomegalovirus (HCMV) in human embryonic lung fibroblast cell cultures. The introduction of the 4-substituent led to a strong reduction of antiherpes activity for the 5-I-dUrd but not for the 5-I-araU analogues, with the exception of 4-O-difluoromethyl-(E)-5-(2-bromovinyl)-araU (CF_2-BrVaraU) which displayed nearly 10 times lower activity against HSV-1, HSV-2 and VZV than the corresponding parent compound BrVaraU. 4-O-difluoromethyl-5-methyl-araU (CF_2-araT) showed a high and selective antiherpes effect comparable with that of 9-(2-hydroxyethoxymethyl)guanine (acyclovir). A lack of activity against HCMV was found for all investigated new 4,5-disubstituted uracil nucleoside derivatives.

R32.40

EFFECTIVE OBSERVATION FOR ANTI-HEPATITIS B VIRUS OF INTERFERON IN ASSOCIATION WITH ACYCLOVIR.....Wu Zhang-qi, Xiong Kai-jun
Wuhan Institute of Virology, Academia Sinica and Tongji Medical University, Wuhan, PR of China.

In this paper, 42 cases with chronic hepatitis B as well as viral carriers were reported, whose ages varies from 16-44 and the courses of disease from half a year to 11 years. They were arbitrarily divided into 3 groups: in the first group, 10 cases were treated with interferon intramuscularly, 60000 , three times a week in a period of 3 months. The result of this group showed that there were 3 cases whose HBeAg had turned negative with anti-HBe turned positive, and DNAP and HBV-DNA had turned negative in other 2 cases. In the second group, 22 cases were treated with acyclovir, intravenously, 15mg/kg, 20 days a month in a period of 60 days. The result was better than that in the first, where there were 6 cases' HBeAg turned negative with anti-HBe turned positive in 5 cases, HBsAg turned negative in 5/11 cases, DNAP turned negative in 9/18 cases and HBV-DNA turned negative in 3/18 cases. In the third group, 9 cases were treated with interferon in association with acyclovir, the result was HBsAg turned negative in 6 cases with anti-HBe turned positive in 5 cases, other viral indexes were similar to the second group, which showed the third group was the best among the three.

R32.41

PLANT REGENERANTS PRODUCTION WITH GRAPEVINE CALLUS SUSPENSION CULTURE AND STUDY ON THIS MODEL DIFFERENT AV PREPARATS. P.G.Kovalenko, L.A.Galkina, A.P.Galkin, S.P.Ponomarenko. Department of Plant Genet.Engineering, Institute of Bioorganical Chemistry, Acad.Sci.of the Ukrainian SSR, Kiev 252660

For studing of the biological activity of different Anti Vir.preparats, more effectively use meristems, protoplasts and suspension cultures. Grape vine callus Cabernet Sauvignon, was obtained from the young stems and leaves, using modified MS medium with 4.5mg/l BA, 2mg/l NAA, 0.33 mg/l GA, sucrose 3%. Primary callus was subcultured during 4 months. With this callus was derivated cell suspension it was cultured on 1/2 fold medium with MS macro and Hellers micro elements, with addition 2g/l CH, 3mg/l BAP, 2mg/l NAA, 3.3mg/l GA, amino acids and sugars. Callus suspension was treatment various concentrations of Virasol2 and synthetical pyri—dine derivates. With callus suspension culture was obtained callus and plant regenerants./Ref., P.G.Kovalenko, B.A.Levenko. Acta Biologica Hungarica, v.37, 1986, p. 278 /. HIgh level of the suspension cultures allow to use them an as effective model for obtain free virus materials in Grapevine.

R33.1

DIFFERENTIAL ACCUMULATION AND CONTROL OF EXPRESSION OF SONCHUS YELLOW NET VIRUS PROTEINS. I.D. Ismail & J.J. Milner, Dept. of Botany, Glasgow University, Glasgow, U.K..

Tobacco infected with Sonchus yellow net virus developes acute symptoms 10 days post-inoculation but undergoes subsequent apparent recovery. After 60 days, plants appear visually identical to healthy controls and show low virus levels. However major ultrastructural differences in the nuclei and chloroplasts are apparent in electron micrographs. Western blots of total cellular proteins reveal the presence of relatively high levels of virus nucleoprotein N and low levels of G protein. Proteins N1 & N2 are not detectable, but a novel antigenically reactive protein of 41kD accumulates. Although these plants are still infectious, mature virus particles are not visible under the electron microscope. Despite the fact that in vivo incorporation of ^{35}S-methionine into viral proteins is not detectable, the poly(A)$^+$RNA fraction contains significant quantities of viral mRNAs, capable of stimulating synthesis of all the major virus proteins in vitro.

R33.2

FUNCTIONAL DOMAINS OF VSV GLYCOPROTEIN AND MATRIX PROTEIN STUDIED BY GENE EXPRESSION. W. Keil, Y. Li, R.M. Snyder, R.R. Wagner. Department of Microbiology, School of Medicine, Box 441, University of Virginia, Charlottesville, VA 22908

Studies are being instituted to map the functional and monoclonal antibody-binding domains of the glycoprotein (G) and matrix (M) protein of vesicular stomatitis virus (VSV). We are particularly interested in the G-protein epitopes that react with neutralizing antibody and the ribonucleoprotein- and membrane-binding sites of the M protein. cDNA's encoding the M and G proteins of VSV were inserted into a plasmid between T7 phage promoter and terminator sequences, which are flanked by sequences of the thymidine kinase gene. The obtained recombinant plasmids were able to direct the insertion of the M and G genes, which are under control of the T7 promoter, into the TK locus of vaccinia virus. Cells transfected with those plasmids or infected with the recombinant vaccinia viruses were coinfected with another vaccinia virus recombinant expressing the T7 RNA polymerase. High levels of expression were obtained and VSV-temperature sensitive mutants, *ts*O23 and *ts*O45 could successfully be rescued at the nonpermissive temperature by the expressed M or G proteins. Moreover up to 3 mg of M protein was produced from 1l cultures of *E. coli* cells transfected with a plasmid derived by insertion of the M gene into expression vector pkk233-2. Expressed G and M proteins and site-directed mutants thereof are being isolated by affinity chromatography using Sepharose 4B coupled polyclonal rabbit antisera directed against M or G protein.

R33.3

IMMUNOCYTOCHEMICAL LOCALIZATION OF VIRAL STRUCTURAL PROTEINS OF POTATO YELLOW DWARF VIRUS IN INFECTED CELLS. 1)Na-Sheng Lin, 2)Yau-Heiu Hsu and Ren-Jong Chiu. 1)Institute of Botany, Academia Sinica, Nankang, Taipei; 2)Agricultural Biotechnology Laboratories, National Chung Hsing University, Taichung, Taiwan

Potato yellow dwarf virus (PYDV) is a member of plant rhabdoviruses with typical bacilliform morphology. An indirect immunogold staining procedure was used to locate the structural proteins of Sanguinolenta isolate (SYDV) of PYDV in infected tobacco cells by using antibody specific to PYDV. Western blots showed this antibody specifically reacted with all five structural proteins of PYDV, e.g. L(190K), G(84K), NC(52K), M_1(31K) and M_2(26K) proteins. Ultrathin sections of glutaraldehyde-fixed, Lowicryl HM 20-embedded PYDV tissues were then immunostained with anti-PYDV serum followed by gold-IgG complexes. Besides over the PYDV virions at the periphery of the nuclei as well as in the cytoplasm, gold particles were found predominantly over the nucleoplasm of infected nuclei. There was no similar deposition of gold particles in the uninfected or healthy control cells. It appears that nucleoplasm associated with PYDV infection is composed largely of viral structural proteins and is apparently the site for viral protein accumulation.

R33.4

EFFECT OF ANALOGS OF S-ADENOSYLMETHIONINE ON IN VITRO POLYADENYLATION BY VESICULAR STOMATITIS VIRUS. D. M. Hunt, Department of Microbiology and Immunology, University of South Carolina School of Medicine, Columbia, SC 29208, U.S.A.

Vesicular stomatitis virus (VSV) virions contain the enzymatic machinery for the synthesis of capped, methylated, polyadenylated mRNAs. Rose, Lodish and Brock reported that S-adenosyl homocysteine [SAH], an analog of the methyl donor, S-adenosyl methionine [SAM], causes increased polyadenylation by VSV in an in vitro transcription system. We have evidence that the viral L protein modulates the extent of this response to SAH. To investigate whether the effect on polyadenylation was associated with SAH being an analog of SAM, or whether it was because of a partial structural similarity between SAH and ATP, the usual substrate for the polyadenylation reaction, we have been examining the effect of various analogs of SAM and ATP on polyadenylation. Those analogs which increase polyadenylation appear to be those which are closely related to SAH and SAM. High concentrations of SAM reverses their effect, suggesting that the effect of SAH and its related analogs on polyadenylation is due to its affinity for a SAM binding site.

R33.5

ECONOMICAL REGIMENS OF SUCKLING MOUSE BRAIN RABIES VACCINE FOR POST-EXPOSURE PROPHYLAXIS

T. O. Harry, A. Nasidi, H. O. Akinlade, A. Adeiga
National Institute for Medical Research, Yaba, Nigeria.

This study was carried out in an effort to reduce the cost of anti-rabies treatment in Nigeria, a country in which rabies is endemic.

Dog-bite victims referred to us were given either: (1) 8 doses (full dose on days 0 to 4 sub-cutaneously, and one-tenth dose intradermally on days 10, 28 and 90); or (2) one-tenth dose at two sites intradermally on each of days 0, 3, 7, 14, 28 and 90. In each regimen vaccine was administered with or without hyperimmune serum on day 0, depending on the history of the case.

All 74 vaccinees survived, with mild and transient side effects in 27.4% of the first group, and 13.0% of the second group. In the first group of vaccinees, 79.4% seroconverted by day 10 (GMT = 3.46 E.U./ml) and 100% by day 28 (GMT = 5.57 E.U./ml). In the second group seroconversion rate was 90.9% by day 14 (GMT = 2.72 E.U./ml) and 100% by day 28 (GMT = 3.11 E.U./ml). Hyperimmune serum on day 0 had no effect on the kinetics of antibody development.

Rabies in the dogs was confirmed in respect of four biting episodes, and strongly suspected in respect of twelve others in which examination was not possible.

R33.6

MOLECULAR CHARACTERIZATION OF DEFECTIVE INTERFERING PARTICLES FROM A HEAT RESISTANT STRAIN OF VSV_{NJ} (OGDEN). E.W. Rud and C.Y. Kang. Department of Microbiology and Immunology, Faculty of Health Sciences, University of Ottawa, Ottawa, Canada.

Several multiple deletion DI particles have been generated from a heat resistant strain of VSV_{NJ} (Ogden). Initially, these DI particles were characterized by hybridizing their genomic RNA's to northern blots of VSV_{NJ} mRNA's. To further define the deletions, southern blots of restiction endonuclease digested clones of VSV_{NJ} mRNA's were hybridized with ^{32}P labeled DI particle genomic RNA's. The DI particle genomic RNA's hybridized to all VSV_{NJ} specific restriction endonuclease fragments. These results indicate that several small deletions, rather than one large deletion, are being generated throughout each gene. We conclude that the "Jumping" polymerase model for DI particle generation would best explain our findings.

Two of these DI particles, NJ-121 and NJ-PG2 contain the template regions for both the positive and negative strand leader RNA's. Both leader RNA's are transcribed when the endogenous RNA transcriptase reaction is carried out in vitro. We are presently determining the location of the template region for each leader RNA in the NJ-121 and NJ-PG2 genomes.

R33.7

SONCHUS YELLOW NET VIRUS: A PLANT RHABDOVIRUS WITH SIX GENES. L. A. Heaton, R. I. Hillman, D. Zuidema, and A. O. Jackson. Department of Plant Pathology, University of California, Berkeley, CA 94720. USA.

Sonchus yellow net virus (SYNV) is a plant rhabdovirus which contains a non-segmented, negative-stranded genome of approximately 13,000 nucleotides (NTs). The genome is transcribed into mRNAs, designated SYNV-complementary (sc) RNAs 1-5, with the potential to encode the five known SYNV structural proteins (N, M2, M1, G, and L). SYNV-specific recombinant DNA clones were derived from both the poly A+ RNA of SYNV-infected tobacco leaves and from SYNV genomic (g) RNA. The cDNA clones have been used to generate antigenic β-galactosidase (β-gal)/SYNV fusion proteins, to detect a previously unreported sixth scRNA, to construct a physical map of the viral genome, and to determine the nucleotide sequences at SYNV gene-junctions. The results of "western blot" analyses indicate that scRNA 3 encodes the N protein, and that scRNA 5 encodes the M2 protein. Comparisons of the coding-capacities of scRNAs 1 and 2 with the estimated molecular weights of L and G suggest that scRNA 1 encodes L and that scRNA 2 encodes G. The coding assignments of scRNAs 4 and 6 are unclear. The gene order of SYNV, based on the results of northern blot analyses and verified by NT sequence data, is 3' N-M2- scRNA4 - scRNA 6 - G-L 5'. Nucleotide sequence data also show that SYNV gene-junction sequences are similar to those of other rhabdoviruses and to several paramyxoviruses.

R33.8

ANTIVIRAL ACTIVITY OF MONOCLONAL ANTIBODIES SPECIFIC FOR INTERNAL PROTEINS OF THE RABIES VIRUS. M. Lafon, Institut Pasteur, Unité de la Rage, Paris, France.

Monoclonal antibodies (MAbs) specific for the rabies N and NS were mechanically introduced into monolayer BHK-21 cells with the scrape-loading technique (Mac Neil et al., 1981). After the cells restored fibroblastic shape, they were infected with rabies virus. Inhibition of infection was monitored by measuring the intra-cytoplasmic viral nucleocapsid with an enzyme immunoassay using anti-N protein rabbit serum and by measuring the release of infectious virus by the plaquing system.

All the MAbs of the three independent antigenic sites on the N protein were able to block the infection with rabies virus. On the contrary only MAbs describing the site I on the NS have an antiviral activity.

If MAbs were introduced into cells already infected, the virus inhibition was complete when anti-N MAb were introduced between 0 and 5 h post infection (pi) and ineffective beyong 9 h pi. The inhibition was dose dependent. These facts suggest that anti-N and some anti NS MAbs can delay the infection by neutralizing newly translated N and NS proteins. The inefficacy of some anti-NS MAb could be explained by their discriminative reactivity with different phosphorylated forms of NS.

R33.9

COMPLETE STRUCTURE OF THE RABIES GENOME: INSIGHT INTO RHABDOVIRUSES EVOLUTION. N. Tordo(1), O. Poch(2), A. Ermine(1), G. Keith(2) et F. Rougeon(1) (1) Institut Pasteur, Paris; (2) Institut de Biologie Moléculaire et Cellulaire, Strasbourg, France.

Rabies virus genome is a single continuous negative strand RNA coding for five viral proteins: N, M1, M2, G et L. Three specific oligodeoxynucleotide primers deduced either from the 3' sequence of the genomic RNA or from sequence studies of proximal rabies cDNAs, were sufficient for walking along the whole rabies genome. The structure of the genome has been recently completed by determining the sequence of the 5' half including the polymerase L and the 5' untranscribed region.

The high level of similarity observed between the polymerases of rabies virus and VSV contrasts with the divergence found between other proteins, except in limited streches of the nucleoprotein N (NAR, 14, 2671-2683, 1986). Such conservation of the polymerase amino acid sequence throughout evolution suggests that the multiple enzymatic activities of this protein (polymerization, phosphorylation, polyadenylation, capping) are essential for Rhabdoviruses multiplication. Similarly, we have observed a conservation of transcription start and stop signals between the two viruses (PNAS, 83, 3914-3918, 1986). This shows that Rhabdoviruses and more generaly unsegmented negative strand RNA viruses, in spite of their high rate of evolution, have conserved the same basic genomic organization, the signal sequences and the enzymatic activities essential for their characteristic mode of multiplication.

R33.10

CHARACTERIZATION OF REVERTANTS OF VESICULAR STOMATITIS VIRUS M PROTEIN MUTANTS. K. Morita and J. Lenard. UMDNJ-Robert Wood Johnson (formerly Rutgers) Medical School, Piscataway, NJ 08854-5634.

Twenty-five spontaneous revertants derived from 4 ts M protein mutants derived from 2 wild type strains (Orsay and Glasgow) of VSV were studied. Each ts mutant differed from its wt by 2-3 amino acids; 13 different revertant sequences were found, 12 of which differed from its parent ts by only 1 amino acid. Three revertant sequences were back mutations in different single amino acids, the rest were pseudoreversions occurring in various positions, accounting for the notably high reversion rate of tsM mutants.

Several properties of M protein are altered in tsM mutants: (i) decreased ability to prevent overproduction of intracellular viral RNA during infection; (ii) decreased inhibition of RNA polymerase activity of purified viral nucleocapsids; (iii) increased intracellular degradation at non-permissive temperature. Of these, (i) is always completely recovered with recovery of the temperature stable phenotype. Revertant M proteins vary with regard to properties (ii) and (iii); some resemble wt M, others resemble tsM, while still others show intermediate behavior. Ability to regulate viral RNA production in infected cells thus appears to be a necessary concomitant of the temperature stable phenotype.

R33.11

GENETIC ANALYSIS OF A HETEROTYPICALLY INTERFERING DEFECTIVE INTERFERING PARTICLE OF VESICULAR STOMATITIS VIRUS CONTAINING THE ENTIRE L GENE. P.K. Kibler and H.R. Thacore, Dept. Microbiology, SUNY at Buffalo, Buffalo, NY 14214

Mapping of a heterotypically interfering defective interfering (DI) particle of a small plaque mutant of vesicular stomatitis virus (spVSV) was conducted using cDNA clones of the entire VSV genome. Results showed that the spVSV DI particle RNA hybridized only with the three cDNA clones comprising the entire L gene. The cDNA LH 679 representing the middle third of the L gene did not hybridize with RNA from a non-heterotypically interfering DI particle. Hybridization of Eco RI/Pst I digested fragments of LH 679 to spVSV DI particle RNA showed all the fragments hybridized. RNA from BHK-21 cells infected with spVSV DI particles was probed for the presence of L gene transcripts by hybridization with a cDNA of the 5' half of the L-gene (L5). The RNA hybridized with the L5 cDNA but not with an N gene cDNA. Cells infected with spVSV DI particles also demonstrated immunofluorescence for the presence of L protein. These results suggest transcriptional activity by the spVSV DI particles. These products may be involved in the spVSV DI particle's ability to interfere heterotypically. Reversion of spVSV to the large plaque phenotype causes loss of its heterotypic interfering ability suggesting that the same mutation affects both the plaque size and interfering ability.

R33.12

FUSION OF SPODOPTERA FRUGIPERDA CELLS BY THE CELL SURFACE EXPRESSION OF BIOLOGICALLY ACTIVE VSV-G PROTEIN USING A BACULOVIRUS EXPRESSION VECTOR.
D.A. McLeod and C.Y. Kang.
Department of Microbiology and Immunology, University of Ottawa School of Medicine, Ottawa, Canada K1H 8M5.

The coding sequences of the Vesicular Stomatitis Virus G protein (VSV-G) were inserted into a baculovirus transfer vector. Spodoptera frugiperda cells were co-transfected with DNAs from the recombinant transfer vector and AcNPV wild type virus. A polyhedrin negative recombinant virus was selected and designated AcNPV-VSV-G. S. frugiperda cells infected with the recombinant virus produced extensive cell fusion by 48 hours post infection. Viral mRNA prepared from the recombinant virus infected cells specifically hybridized to VSV-G specific DNA probe. VSV-G protein was identified by immunoprecipitation using monospecific VSV-G antibody. The cell surface location of the VSV-G protein has been confirmed by immuno-electron microscopy. This data supports our previous finding that cell surface expression of VSV-G protein causes cell fusion.

R33.13

USE OF AN IN VITRO SYSTEM TO ASSEMBLE ISOLATED VESICULAR STOMATITIS VIRAL RNA INTO NUCLEOCAPSIDS. Beloo Mirakhur and Richard Peluso, Thomas Jefferson University, Phila. PA 19107.

Replication and encapsidation of vesicular stomatitis virus (VSV) genome RNA are coupled processes in vivo. We have developed an in vitro system in which we can study the process of VSV genomic encapsidation in the absence of ongoing genomic replication. This system consists of cytoplasmic extracts prepared from wild type (WT) infected cells as a source of viral proteins. These cytoplasmic extracts have been previously shown to support in vitro replication of endogenous WT and defective interfering particles (DI) RNA at comparable rates to an in vivo system. 5' end labeled DI RNA prepared from purified DI particles is added to the cytoplasmic extracts of either VSV infected or mock infected cells and analyzed for encapsidation. Encapsidation is assayed for by development of resistance to RNAse and a decrease in buoyant density to 1.33g/ml. Results show that encapsidation can occur in vitro and that this is a viral protein specific process in that incubation with infected cell extracts results in the formation of a nucleocapsid structure. Extracts of uninfected cells are not able to support DI RNA encapsidation. This system is being used to answer the question whether encapsidation in an in vitro system results in the generation of an infectious particle, and to determine nucleotide sequences which may be acting to initiate nucleocapsid assembly.

R33.14

KINETIC AND QUANTITATIVE ANALYSIS OF MULTIPLE FORMS OF THE VSV N PROTEIN IN INFECTED CELLS. Richard Peluso, Thomas Jefferson University, Philadelphia, PA 19107

Previous work from our laboratory has demonstrated that the nucleocapsid protein (N) of vesicular stomatitis virus (VSV) is present in several forms in infected cells. One form is complexed with the viral NS protein in a 1:1 molar ratio, and a second form is not complexed with the NS protein and exhibits a higher sedimentation rate than the complex. We have performed a series of experiments to analyze the relationship between these forms of the N protein. Pulse and chase experiments demonstrate that the N protein is made first as the form which binds to the NS protein, and that with increasing times of chase it is either assembled into nucleocapsids or is converted to two higher sedimenting forms, neither of which appear to be complexed with the NS protein. Activity of these forms has been assessed, with only the N/NS complex demonstrating the ability to support the replication of viral nucleocapsids. Using a newly developed quantitative immunoblotting procedure, we have quantitated the three differentially sedimenting species of the N protein, and have shown that at later times post infection (6-7hr), the faster sedimenting, uncomplexed form of the N protein accounts for as much as 50% of the soluble N protein in the cell. The role of these various N protein species in VSV replication is being investigated.

R33.15

WHEN ARE DESCENDANTS OF A COMMON PROGENITOR SUFFICIENTLY DIFFERENTIATED TO BE CLASSIFIED AS A VIRUS FAMILY? M.P. KILEY.....Division of Viral Diseases, Centers for Disease Control, Atlanta, GA 30333, U.S.A.

Our studies with Marburg and Ebola viruses have defined a pair of viruses with characteristics not shared by any known virus family. They are enveloped, negative strand RNA viruses with a monopartite genome. They are most likely descended from the same common progenitor as the paramyxo- and rhabdoviruses. They share common features with these viruses such as; general structure, single strand RNA genome, internal nucleocapsid and certain replication signals. There are also characteristics that are different from the other two families; specific morphology, virion and RNA size and the number and location of viral proteins. A comparison of this new virus family, the Filoviridae, with the similar families, the Paramyxoviridae and Rhabdoviridae, will be beneficial to taxonomists. An analysis of similarities and differences among these virus families will serve as the focus for discussion of the importance of various virus characteristics to viral taxonomy.

R33.16

COMPARATIVE ANTIGENIC STRUCTURE OF THE GLYCOPROTEIN FROM THE POLISH BAT STRAIN OF RABIES VIRUS. W. Wunner, J. Larson, B. Dietzschold. The Wistar Institute, Philadelphia, U.S.A.

A street rabies virus strain which was isolated from a bat in Poland and diagnosed by a panel of anti-glycoprotein and anti-nucleocapsid monoclonal antibodies as being similar to the Duvenhage strain originally isolated in Africa was analyzed at the molecular level and compared to the known antigenic structure of the glycoprotein of fixed rabies virus strains. Comparisons of nucleotide and deduced amino acid sequences derived from the glycoprotein gene of the Polish bat strain with the corresponding sequences from several fixed rabies virus strains have revealed a direct correlation between sequence homologies and the number of unshared epitopes. Antigenic distinctions appear to be greatest between several bat strains in Europe and the rabies virus strains isolated from European terrestrial animals. Conserved regions and locations of altered epitopes of the Polish bat glycoprotein as compared to epitopes in antigenic sites I, II and III of fixed rabies virus glycoprotein will be discussed.

R33.17

MECHANISMS OF RABIES VIRUS NEUTRALIZATION BY GLYCOPROTEIN-SPECIFIC MONOCLONAL ANTIBODIES. B. Dietzschold, M. Tollis, M. Lafon, W. Wunner. The Wistar Institute, Philadelphia, PA.

Incubation of radiolabeled rabies virus with neutralizing monoclonal antibodies (MAbs) resulted in complete neutralization of the virus but only partial inhibition of virus binding to and internalization by BHK cells. Several of the neutralizing MAbs were capable of preventing infection after virus adsorption to the cell; up to 30% of the bound virus was released when cells containing adsorbed virus were incubated with these MAbs, indicating that the release of bound virus accounts only in part for the neutralization of adsorbed virus. Temperature shift experiments using rabies virus-infected BHK cells indicate that the virus is endocytosed within 4 h after infection. Treatment of infected cells with neutralizing MAbs had no effect on virus uptake at 37°C and the MAbs were endocytosed together with the virus. The ability of some MAbs to neutralize adsorbed virus strongly correlates with their ability to inhibit fusion catalyzed at pH 5.8 of rabies virus-infected cells. These data suggest that some of the MAbs can neutralize rabies virus by inhibiting the intraendosomal acid-catalyzed fusion step.

R33.18

MOLECULAR EPIDEMIOLOGY AND EVOLUTION OF VESICULAR STOMATITIS VIRUS. S.T. Nichol. School of Veterinary Medicine, Univ. of Nevada, Reno, U.S.A.

Vesicular stomatitis virus (VSV) infects cattle, horses, swine, and occasionally humans, throughout the Americas. Some regions (e.g. the United States), experience periodic and unpredictable disease epidemics, whereas other regions (e.g. Central America) have extensive endemic virus activity. VSV isolates from the United States, Mexico, and Central America, isolated during 1982 to 1986, are being analyzed by T1 RNase fingerprinting and sequencing of virus RNA genomes. The results demonstrate that virus outbreaks in Central America are unrelated to those in Mexico and the U.S. In addition, much greater genetic diversity appears to exist among virus isolates from endemic regions than from epidemic regions. Close similarities are observed between the T1 fingerprints of U.S. and Mexican VSV isolates suggesting that Mexico may be the source of virus initiating VSV epidemics in the U.S. The natural variation of these viruses appears to be considerable, suggesting that VSV evolution during natural infections may be as high as has been demonstrated for these viruses under laboratory conditions.

R33.19

VESICULAR STOMATITIS VIRUS MEMBRANE PROTEIN CAN BIND TO RNA IN VITRO.

J. Perrault and A. Haller. Department of Biology and Molecular Biology Institute, San Diego State University, San Diego, California 92182, USA

We have explored RNA binding properties of VSV proteins by probing Western blots with P32-labelled VSV RNA. Surprisingly, the membrane M protein and not the core-associated N protein shows strong binding under these conditions. Binding is abolished at physiological salt and pH conditions in the absence of magnesium but is retained in its presence. Ribosomal RNA and double-stranded DNA also appear to bind indicating little specifity for the type of nucleic acid which can bind. Mild trypsin treatment of detergent-treated virions which selectively digests the lysine and arginine rich amino terminal segment of the M protein has no effect on the binding ability of the remainder of the protein. We conclude that the VSV M protein has the potential to bind nucleic acids in vivo and that this property may play an important role in the inhibition of viral RNA synthesis mediated by this protein. Further characterization of this phenomenon will be described.

R33.20

TEMPLATE JUMPING BY VESICULAR STOMATITIS VIRUS POLYMERASE DURING IN VITRO TRANSCRIPTION. W. Helfman and J. Perrault, Department of Biology and Molecular Biology Institute, San Diego State University, San Diego, California 92182, USA.

The addition of purified standard vesicular stomatitis virus (VSV) template (N protein-RNA complex free of polymerase) to detergent-disrupted VSV defective interfering (DI) particles resulted in a five to ten-fold stimulation of RNA synthesis, accompanied by the production of plus strand leader RNA. The latter was assayed by solution hybridization to a 3'-end-labeled probe derived from VSV standard genome RNA. Efficient synthesis of plus strand leader RNA occurred whether or not the mixture was preincubated at the high ionic strength reportedly necessary for dissociation of VSV transcriptase from its template. We therefore conclude that in vitro transcription conditions allow the VSV polymerase to dissociate from its template and initiate synthesis on a different one. Further characterization of the "jumping" of DI-derived transcriptase to a recipient standard virus template will be reported.

R33.21

MORPHOLOGY STUDY ON FIXED RABIES VIRUS (BEIJING STRAIN) IN BHK21 CELLS BY ELECTRON MICROSCOPY Xie Bin, Li De-shong, Viral Laboratory & Electron microscope laboratory, Hong-shan Hospital, Wuchang, China. Lin Pang-tao, Wuhan Institute for Biological Products, Wuchang, China.

The BHK21 cells infected with the Beijing strain of fixed rabies virus have been studied by electron microscopy. Thin sections of infected cells reveal cytoplasmic spherical and elongated particles usually associated with masses of homogeneous material. Most of them were seen (1) multiplying in the enlarged cavities of SER, RER, (2) protruding from their membranes toward the cavities, (3) some budding from the cell membranes, and (4) some free particles in extracellular spaces. Except the morphogenesis described above, viral maturation was also observed in the nucleous membranes and entire particles in perinucleous spaces. Some of them were closed to inner membranes of the nucleous. The morphogenesis above was also seen in infected chicken embryo cells with Flury strain. To compare with the mophogenesis of Flury strain, the morphological observation of Beijing strain of rabies virus was very similar to that in chicken embryo cells. The particles were not seen in uninfected cultural cells.

R34.1

PARVOVIRUS B19 INFECTION IN ADULTS: CONTROLLED EPIDEMIOLOGICAL STUDY OF MANIFESTATIONS AND FACTORS DETERMINING OUTCOME. *A Woolf, G Campion, ¥P Klouda, A Chiswick, †B Cohen, P Diappa. *Royal Nat. Hosp. for Rheum. Diseases, Bath; Rheumatology Unit, Bristol Royal Infirmary; ¥UK Transplant Service, Bristol; †Virus Ref Lab, Colindale, London, UK.

To define the usual adult manifestations of B19 infection and factors that determine them we have studied all adults (cases and non cases) associated with a recent outbreak at a primary school of B19 symptomatically affecting 53% of children. 454 of 475 adults were interviewed and blood obtained for serological diagnosis. 54 cases (22M, 32F, age 37±5) of recent infection were identified (B19 specific IgM by RIA). Non-cases acted as controls. Cases were also HLA-A,B,C and DR typed.

14 cases were asymptomatic (6M 8F); 26 (5M 21F) had an acute onset symmetrical polyarthropathy lasting 2 days-7 months (median 10 days): 32 a flu like illness; and 23 an erythematous rash, affecting the face in 4; 3 arthropathy alone. Women usually had rash with arthropathy, men a flu like illness. In B19 specific IgG and IgM-ve controls the incidence of joint symptoms with rash was 1% (cases 35%). The frequency of HLA-A, B and C antigens was similar to controls irrespective of clinical outcome. DR1 was present in 14.8% of cases (20.1% controls) but absent in those with arthropathy persisting > 2 months, as noted in 27 other selected persistent cases.

In a controlled study arthropathy was a common outcome in adult women. DR1 may be protective against its persistence.

R34.2

EFFECTS OF INTRAUTERINE INFECTION WITH THE HUMAN PARVOVIRUS B19. A.M.W. van Elsacker-Niele, M.M.H. Salimans, Chr. Vermey-Keers, M.J. Anderson, G.J. Fleuren, J. Versteeg, H.T. Weiland Depts. of Virology, Embryology and Pathology, Faculty of Medicine, University of Leiden, Holland, and Dept. of Medical Microbiology, Faculty of Clinical Sciences, University College London and Middlesex Hospital Medical School, London, U.K.

The common clinical manifestation of infection with the human parvovirus B19 is erythema infectiosum. In patients with chronic haemaolytic anaemia this infection can cause aplastic crisis. Since 1984 this virus has been recognized as a possible cause of spontaneous abortion and intrauterine fetal death. In the U.K. as well as in The Netherlands efforts are being made to determine just what the incidence of parvovirus B19-induced abortions and intrauterine fetal deaths is. These studies have already resulted in the detection of several cases of infection with the human parvovirus B19 in pregnancy. A number of these cases ended in abortion or intrauterine fetal death, and extensive serological, virological, embryological and pathological studies were performed. Investigators have been able to detect the virus in fetal and/or placental tissues on more than one occasion, and cases of hydrops fetalis and one case of microphthalmia in an aborted fetus are reported.

Whether this virus can cause congenital malformations or is too fetotxic to do so, will hopefully be elucidated by the studies mentioned above.

R34.3

IN VITRO ENCAPSIDATION OF MINUTE VIRUS OF MICE DNA: EVIDENCE FOR A PACKAGING SIGNAL IN THE VIRAL GENOME AND PARTIAL PURIFICATION OF ENCAPSIDATION PROTEINS. K. Brudzynska, R. Salvino, E.A. Faust. Department of Biochemistry, Faculty of Medicine, University of Western Ontario, London, Canada, N6A 5B7.

A system for in vitro encapsidation of minute virus of mice (MVM) DNA has been developed in which proteins with sequence-specific DNA binding properties catalyze the conversion of a linear duplex DNA substrate to ssDNA. The conversion from duplex DNA to ssDNA does not require ATP. Using plasmid constructs with defined subsets of 3' and 5' terminal viral sequences, as well as the entire MVM genome as substrates, we established that the reaction was strictly dependent on a 910 base pair 5'-terminal segment of MVM DNA. The encapsidated ssDNA was approximately 5.0kb for plasmid substrate ranging in size from 3.3 to 8.7 kilobase pairs. The proteins(s) responsible for the reaction were present only in extracts from MVM-infected cells. Uninfected cells were devoid of encapsidation activity. To elucidate the nature of the protein(s) involved a cytoplasmic extract from MVM-infected Ehrlich ascites cells was fractionated by differential centrifugation and column chromatography. Initial results indicated that a soluble host cell factor(s) combined with empty viral capsids recognizes a specific DNA sequence near the 5' end of the viral genome and initiates the encapsidation reaction.

R34.4

MORPHOLOGICAL AND BIOLOGICAL CHARACTERISTICS OF CANINE PARVOVIRUSES. D.J. Li, C.M. Hu, Z.H. Xi, C.G. Peng. Academy of Military Medical Sciences, Beijing, China.

Canine parvoviruses were isolated from fecal samples of dogs with vomiting and hemorrhagic diarrhea. These viruses could reproduce in primary feline kidney cell cultures and agglutinate porcine, monkey or cat erythrocytes. Electronmicroscopy of the infected cells revealed the virus particles (18-26nm) and electron dense intranuclear inclusions. The specificity of these structures was testified by immune ultramicroscopy and avidin-biotin immunoperoxidase staining.

R34.5

MATERNAL SERUM α-FETOPROTEIN - A MARKER OF FETAL APLASTIC CRISIS DURING INTRAUTERINE HUMAN PARVOVIRUS INFECTION. D. Carrington*†, D.H. Gilmore†, M.J. Whittle†, D. Aitken†, A.A.M. Gibson†, W.J.A. Patrick†, T. Brown**, E.O. Caul††, A.N. Field‡, J.P. Clewley‡, B.J. Cohen‡. *Regional Virus Laboratory, Ruchill Hospital, †Royal Hospital for Sick Children, Glasgow, Scotland, **University of Aberdeen, Aberdeen, Scotland, ††PHLS, Bristol, England, ‡PHLS, Colindale, London, England.

Human parvovirus B19 infection with minimal symptoms during the second trimester of pregnancy led to the development of hydrops fetalis and intra-uterine death in 2 cases. Maternal serum α-fetoprotein levels were raised in both patients and predated the ultrasonic detection of hydropic features, providing a sensitive marker of early fetal involvement. Fetal blood sampling in one case revealed the features of aplastic crisis.

In a retrospective study of a further 3 affected and 11 unaffected cases of B19 infection during pregnancy, a raised maternal serum α-fetoprotein level correlated with a 'worse prognosis' for the affected pregnancies with the subsequent development of hydrops fetalis.

Clinical, virological and histopathological features of the two affected fetuses will be presented.

R34.6

ATTENUATION IN THE REGULATION OF GENE EXPRESSION IN SV40, AD-2 AND MINUTE VIRUS OF MICE (MVM). Y. Aloni, M. Kessler, O. Resnekov, M. Seiberg, E. Ben-Gal and O. Amster-Choder. Department of Genetics, Weizmann Institute of Science, Rehovot, Israel.

We shall present evidence for premature termination (attenuation) during transcription of SV40, Ad-2 major late transcription unit and MVM P-4 transcription unit. Attenuation was observed at the same sites in vivo, in isolated nuclei and in vitro using whole cell extract. The attenuation sites were mapped in SV40 94 nt and 155 nt, in AD-2 185 nt and in MVM 142 nt downstream from the transcription start sites. The common feature of the three systems is that the attenuated RNA can be folded into hairpin structure followed by U-residues, Our experiments support the following model: a) The hairpin structure leads to a pause by the RNA polymerase and b) U-rich sequence 3' to such secondary structures facilitates dissociation of the transcript from the template. Other experiments indicate that termination and readthrough factors are involved in regulating termination at the attenuation sites. The readthrough factor has been partially purified.

The biological significance of attenuation in the life cycles of these viruses will be discussed.

R34.7

HUMAN PARVOVIRUS B19: RESTRICTION ENZYME ANALYSIS

J. Mori and B.J. Cohen. Virus Reference Laboratory, Central Public Health Laboratory, London, NW9 5HT, U.K.

Infection with human parvovirus B19 causes a spectrum of disease including asymptomatic infection, erythema infectiosum, acute arthropathy, acute aplastic anaemia and fetal death. It is not known whether strain variation might be responsible for these different outcomes to infection.

All B19 isolates appear to be antigenically very similar. It was therefore decided to look for other evidence of strain variation by restriction enzyme analysis of B19 DNA. Viral DNA was prepared from over 40 sera containing B19 virus and digested with up to 13 restriction enzymes. Though variations in restriction enzyme sites were found, some isolates were identical and could be grouped together. Other isolates had unique restriction enzyme sites and could not be assigned to groups.

The grouping of B19 isolates did not correlate with clinical manifestations of infection. It did, however, reflect the geographical location and year in which the isolates were made.

R34.8

STUDIES ON NONSTRUCTURAL PROTEINS OF FELINE PANLEUKOPENIA VIRUS.

D. Clemens and J. Carlson, Department of Microbiology, Colorado State University, Fort Collins, Colorado 80523, USA.

The long leftward open reading frame of feline panleukopenia virus (FPV) has been expressed in Escherichia coli as fusion proteins. These proteins were bound in western blot by antisera from dogs that have recovered from CPV infection. Rabbit antiserum to one of these fusion proteins bound two nonstructural proteins in FPV infected feline cells with molecular sizes of 84,000 and 60,000 daltons. There was no antigenic cross-reactivity between these proteins and the FPV capsid proteins. The nonstructural proteins appear early in infection and persist throughout the infection. Preliminary cell fractionation experiments suggest that they are present in both nuclei and cytoplasm of infected cells.

R34.9

IDENTIFICATION OF VIRUS ISOLATES FROM FOODBORNE OUTBREAKS.

G. S. Fout, L. C. Osborne & E. P. LARKIN. Virology Branch, DH, FDA, Cincinnati, Ohio, U.S.A. 45226.

Viruses cannot replicate in processed foods. Viruses causing foodborne outbreaks are usually present at very low levels in contaminated foods and may also be present at low levels in clinical specimens from patients with gastroenteritis. Thus, most evidence implicating viruses in foodborne outbreaks is based on epidemiological considerations. Proper specimens from outbreaks, including contaminated food sources, are usually not available for virus identification. Nevertheless, it seems certain that hepatitis A, Norwalk-like viruses, and enteroviruses are transmitted through foods. Astroviruses, caliciviruses, enteric adenoviruses, and parvoviruses may also be food-transmitted.

Enzyme-linked immunoassays, nucleic acid hybridization with synthetic oligonucleotide probes and restriction endonuclease techniques were used to examine fecal samples and virus isolates from fecal samples derived from two shell fish-associated outbreaks for the presence of Norwalk virus and parvoviruses. Parvovirus H-1-like viruses were identified in a number of the specimens and no Norwalk viruses were detected.

R34.10

PARVOVIRUS INFECTION IN BLUE FOX IN NORWAY, A SEROSURVEY.

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Parvovirus infection has recently been associated with reproduction problems in blue fox in Finland. In Norway a small serosurvey was carried out to elucidate the existence of seropositive blue foxes.

A total of 243 serum samples were collected from 16 farms in 3 different districts. These farms had a total of about 2000 breeding females. Samples were selected from females with poor reproduction, i.e. from those that were empty or had small litters in the 1986 breeding season. The hemagglutination-inhibition test was carried out in microplates (4°C) using raccoon dog (Finn-raccoon) parvovirus antigen and green monkey erythrocytes.

Seven of the 16 tested farms had seropositive females with percentages ranging from 8 to 100; the overall percentage was 17. In this material there was no definite evidence of an association between parvovirus infection and poor reproduction results.

R34.11

UPTAKE OF PORCINE PARVOVIRUS BY PERMISSIVE AND NONPERMISSIVE CELLS.

J. F. Ridpath and W. L. Mengeling. USDA-ARS-National Animal Disease Center, Ames, Iowa, USA.

The purpose of this study was to determine if the selective replication of an autonomous parvovirus is a function of specific viral receptors on the cell surface or intracellular factors. The uptake of porcine parvovirus (PPV) by asynchronous cultures of both permissive (porcine derived) and nonpermissive (bovine derived) cells were examined by immunofluorescence microscopy (FA), dual parameter flow cytometry and autoradiography. Both types of cells took up virus as evidenced by positive fluorescence in the cytoplasm 2 h post infection (PI). Fluorescent staining of the nucleus, indicative of viral replication, was only seen in permissive cell cultures 16 h PI. Flow cytometry performed 2 h PI revealed that the percentage of cells taking up virus was actually higher in nonpermissive cells. Uptake of ^{35}S labeled virions indicated that both cell types selectively took up the activated rather than the nonactivated form of the virus. FA observation of cells transfected with replicative form PPV DNA demonstrated that only permissive cells accumulated viral proteins. These findings suggest that while the uptake of PPV in both cell types appears to be an active, selective process, the selective replication of the virus is determined by intracellular factors.

R34.12

DETECTION OF PARVOVIRUS B19 DNA, ANTIGEN AND PARTICLES IN THE HUMAN FETUS.

J.P. Clewley, B.J. Cohen and Anne M. Field. Central Public Health Laboratory, Colindale, London, U.K.

Human parvovirus B19 commonly infects children, causing erythema infectiosum (fifth disease). However, there is a significant adult population which has not been exposed to the virus and, consequently, does not have protective antibody. Recent reports have associated B19 infection during pregnancy with fetal death, although normal outcome of pregnancy is more common.

The role of B19 infection in fetal deaths has been further characterised in a series of laboratory investigation undertaken on tissues obtained at autopsy. These have demonstrated the presence of virion sized DNA by Southern blotting, viral antigen by radioimmunoassay, and viral particles by electron microscopy, all from tissues of hydrops fetalis. These data confirm that the human parvovirus B19 can cross the placenta and replicate in fetal tissues.

R35.1

HAEMORRHAGIC FEVERS IN THE CENTRAL AFRICAN REPUBLIC
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The haemorrhagic fever viruses (HFV) often cause outbreacks of severe human disease and are considered important public health threats througouht subsaharian Africa. Long term seroepidemiological, clinical surveillance, and ecological studies were conducted to evaluate the epidemic risk in the Central African Republic (CAR). Striking levels of Ebola virus, Marburg virus, Rift Valley fever virus (RVFV), Congo-Crimean haemorrhagic fever virus (CCHFV) and Hantaan virus reactive antibody were demonstrated in human population living in divers ecological habitats suggesting widespread activity. Interestingly, few Lassa virus specific antibody seropositives were identified implying that Lassa virus infections are limited or absent from the CAR. Though the serosuvey results indicate endemic HFV activity, but few human cases were observed. RVFV, CCHFV, Hantaan virus and West Nile virus (WNV) were occasionally associated with clinical disease. WNV involvement in fatal haemorrhagic and hepathonephritic disease was unexpected; traditionally, WNV has not been considered as HFV. Collectively, the results seem inconsistant in light og the highly pathogenic nature of African HFV. However, this contradition may be explained by recent findings suggesting the HFV ecological niche may be occupied by less virulent cross reacting virus strains.

R35.2

ESTABLISHMENT OF RISK FACTORS FOR DENGUE VIRUS INFECTIONS
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A prospective epidemiological study of dengue in Malaysia has identified a number of risk factors for dengue virus infection. A seroconversion rate of 5-6% was obtained among a group of cohort children bled every 6 months. Data on socio-demography, behavioural, knowledge, attitude and practice, environmental and ecological aspects revealed that dengue antibody prevalences among these children were significantly higher in the low socio-economic group as well as among children from homes which did not have good knowledge of the vectors responsible for transmission of the disease. Most of them practised water storage but inadequate measures were taken to prevent mosquito breeding. However, antibody prevalences were lower in areas with greater number of animals (mostly chickens) per house.

R35.3

SURVIVAL OF ENTEROVIRUS 70 ON NON-POROUS INANIMATE SURFACES.
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Enterovirus 70 (EV70) has caused at least two major pandemics of acute hemorrhagic conjunctivitis (AHC) in the past 18 years, with the largest number of cases occurring in the coastal areas of the tropics. How this virus spreads and how well it survives in the environment is not known. However, fomites are believed to play a role in its transmission. We examined EV70 survival on stainless steel disks. Each disk (1 cm diameter) received 10 uL of the virus in phosphate buffered saline and was kept at 20±1°C under low (20±5%), medium (50±5%) or high (80±5%) relative humidity (RH). The virus was eluted from the disks with tryptose phosphate broth and the eluate plaque assayed in LLC-MK$_2$ cells. The virus inactivation rate (Ki) was 0.74 $\log_{10}$/hour at the high RH. At the medium and low RH, the loss in virus titre occurred twice as rapidly. To simulate climatic conditions in EV70 epidemic areas, its survival was also tested at 33°C and high RH. Under these conditions, the rate of virus inactivation was similar to that seen at 20±1°C under low or medium RH. This behavior of EV70 appears to be typical of other picornaviruses tested and may explain the geographic distribution and climatic pattern of AHC epidemics due to EV70.

R35.4

FLAVIVIRUS INFECTIONS IN NORTHERN THAILAND. A. Igarashi, T. Fukunaga, H. Goto, T. Ogata, N. Fujita, A. Mori, H. Shishido, K. Bundo, T. Ishimine, O. Charoensook, J. Supawadee, S. Peerakome, N. Maneekarn, V. Vithayasai, P. Limsukon, P. Chiowanich, W. Choochote, C. Khamboonruang, C. Promkuthao, P. Notanonda, V. Rojanaeob, W. Bhruksasri. Nagasaki University, Ryukyu University, Obihoro University, Teikyo University, Kobe University, Japan, & Ministry of Public Health, Bangkok, Chiang Mai University, Thailand.

Antibody prevalence against Japanese encephalitis (JE) and dengue viruses among humans and swine was investigated at various altitudes around Chiang Mai, Northern Thailand, together with virus isolation from field-caught mosquitoes and clinical specimens of encephalitis, dengue haemorrhagic fever (DHF) and related diseases. Antibody positive rate against JE was low both in humans and swine at 2 sampling sites above 1200 meters from sea level, in contrast to 5 places below 500 meters where the antibody positive rate was not uniformly high. Dengue antibody was more prevalent at a sampling place in the center of Chiang Mai Valley in contrast to 6 other sampling places on the hill sides and in Maekong Valley. Six strains of JE virus were isolated from Culex tritaeniorhynchus, Cx. gelidus, and Cx. fuscocephala captured at pigpens in lowlands. While type 2 and 3 dengue viruses were isolated from sera of DHF cases. Some of the encephalitis cases appeared to be dengue encephalopathy as shown by IgM-ELISA and virus isolation.

R35.5

MONOVALENT NEONATAL POLIO IMMUNIZATION. STRATEGY FOR THE DEVELOPING WORLD. B.D. Schoub, S. Johnson, J. McAnerney, L. Gilbertson, K.I.M. Klaassen. National Institute for Virology & Department of Virology, University of Witwatersrand, Private Bag X4, Sandringham 2131, South Africa.

Poliomyelitis immunization strategies in tropical and Third World countries need to be urgently reviewed in view of sub-optimal immunization efficacies achieved with Western immunization schedules. While the WHO has recommended routine supplementary neonatal immunization with trivalent oral poliomyelitis vaccine (TOPV) we have investigated the advantages of supplemental neonatal immunization with monovalent oral type 1 vaccine (MOPV) as type 1 is the major cause of poliomyelitis epidemics in developing countries. A total of 446 neonates was divided into three cohorts receiving respectively, at birth, MOPV (153), TOPV (147) and placebo (146) and studied prospectively for one year. All were given regular TOPV immunization at 3, 4 1/2 and 6 months. Type 1 seroresponse to neonatal immunization was significantly better with MOPV as compared to TOPV - 18.1% versus 14.6% respectively at 1 month and 44% versus 10% respectively at 3 months. Titre drop-off was also significantly less in the MOPV group - 8.5% versus 25.8% for TOPV and 61.4% for placebo at 1 month and 22% versus 72.5% and 88.0% for MOPV, TOPV and placebo respectively at 3 months. Similar advantages with type 1 protective titres were also demonstrated. This study confirms the advantages of supplementary routine immunization with MOPV at birth.

R35.6

PEPTIDE MAPPING OF VARIOUS VIRULENT AND AVIRULENT STRAINS OF EASTERN EQUINE ENCEPHALITIS VIRUS (EEEV). R. Walder, M. Mas. Instituto Venezolano de Investigaciones Científicas, Centro de Microbiología y Biología Celular, Laboratorio de Virus Animales, Apartado 21827, Caracas, Venezuela.

Virion structural proteins (E1, E2 and CP) representing ten different EEEC strains and serotypes, from North & South America, were compared by limitted proteolysis with Staphylococcus aureus V8 protease followed by SDS-PAGE of the resulting cleavage product.

The peptide patterns of the Core non-glycosilated protein (CP) derived from the ten strains were nearly identical, which demonstrated a marked structural conservation. In contrast, the peptide patterns of the mayor structural glycoprotein, E1 (55,000 daltons gp55) were quite heterogeneous and among which most variations occured. Five distinct peptide mapping groups were identified, with no-correlation to serotypes and virulence. On the other hand, also three distict groups were obtained by mapping the minor structural glycoprotein, E2 (52,000 daltons gp52) showing as well strain-dependent differences in their peptide maps with good correlation to serotypes and virulence. Our results suggest that the different EEEV strains show structural changes and were localized mainly on the E1 gp but also on the E2 gp. The gp55 (E1) being associated with both hemagglutination and virus neutralization polypeptide (epitope) is indicative that preferential changes are linked to HA and/or VN protein.

R35.7

CONCURRENT DERMATOPHILOSIS AND LUMPY SKIN DISEASE (LSD) VIRUS INFECTION IN CATTLE - SEROLOGIC EVIDENCE.

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Streptothricosis (Dermatophilosis) caused by Dermatophilus congolensis, (DC) is a severe and debilitating disease of cattle in Nigeria with serious economic implications. It ranks first in importance amongst the various skin infections of cattle and may develop and extend in a dramatic fashion in animals whose resistance to it is modified by intercurrent disease(s). The possible involvement of Lumpy Skin disease virus (LSDV) on the pathogenesis of streptothricosis was investigated following field observations which suggested a possible association between the agents of the two diseases.

Sera collected from herds suffering from clinical dermatophilosis were examined for the presence of antibodies to DC and LSDV using counterimmunoelectrophoresis technique. Out of 502 sera screened 217 (43%) were positive for DC while 224 out of 481 (47%) had detectable LSDV antibodies. Amongst the 217 DC positive samples, 114 (53%) had LSDV antibody titres.

This study therefore suggests a probable role for LSDV in the pathogenesis of dermatophilosis.

R35.8

SEROEPIDEMIOLOGICAL STUDIES OF INFECTIONS WITH SOME NEUROTROPIC VIRUSES IN KARACHI, PAKISTAN IN THE YEARS OF 1983 AND 1985.

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In 1983 to 1985, some probable cases of Japanese encephalitis(JE) were observed in Karachi, Pakistan. Seroepidemiological studies were carried out to know the background and the size of infections among Pakistani persons with JE JE and other flaviviruses (West Nile(WN) and dengue), and with other neurotropic viruses (measles(M), rubella(R), Herpes simplex-1(HS-1) and entero-70(E-70)) in 1983 (280 of healthy persons and out-patients without encephalitides) and 1985 (151 paired serum samples from healthy persons) by measuring antibodies of HI, NT and ELISA.

Among healthy persons, around 50% of them had antibodies against the three flaviviruses, and the most prevalent infection occurred with WNV. In 1983, among the three flaviviruses, significant elevation of antibody positive rate was observed against JE virus only in a group of out-patients but not in healthy persons.

Among all the subjects in both years, antibodies were detected over 80% against M and R, around 70% against E-70 and over 60% against HS-1 viruses.

R35.9

DELTA HEPATITIS VIRUS INFECTION IN ETHIOPIA

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566 HBsAg positive sera from asymptomatic carriers in Ethiopia were tested for the presence of antibodies to Hepatitis Delta Virus (HDV) and HDV Ag. The samples were collected in a large seroepidemiological hepatitis B virus (HBV) study, performed on 5270 recruits.

Overall anti HDV prevalence was 5.8%. The prevalence increases with age within the available range (18-30 years) while HBsAg prevalence is steady. Differences were observed among the 14 administrative regions and among the Ethnic groups. The prevalence in Tigre groups was higher than in the others.

The distribution of HBV markers in anti HDV positive and anti HDV negative individuals was similar. However the presence of HBV-DNA was lower in anti HDV positive sera. All but one of 12 individuals retested after 6 months resulted still positive. In one case seroconvertion from HBeAg to HBe positivity and HBV-DNA clearance were observed.

R35.10

VIRAL ETIOLOGY OF ACUTE RESPIRATORY INFECTIONS AMONG SOUTH INDIAN CHILDREN.

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In a study on the etiology of acute respiratory infections (ARI), children under five years of age were investigated.

Nasopharyngeal aspirates(NPA) and throat swabs were cultured in primary monkey kidney and HEp-2 cell cultures. NPA smears were tested for antigens of five respiratory viruses by indirect immunofluorescence test (IFT).

During Feb.85 to Nov.86, 97(27%) viral isolates were obtained from 364 cases of upper respiratory infection and 68(29%) from 231 cases of lower respiratory infections. 45 per cent of the viral isolates were hemadsorbing agents and 37 per cent were Respiratory syncytial viruses(RSV). RSV presented as an outbreak from August to November. During this period IFT on NPA identified RSV in an additional 20 (13.5%) cases. These findings indicate that in southern India, though tropical in climate, RSV has a seasonal prevalence similar to that reported from the temperate regions of the country.

R35.11

A SURVEY OF ARBOVIRUSES IN SRI-LANKA

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A survey of arboviruses by seroepidemiology and virus isolation from field caught mosquitoes is in progress.The seroepidemiological survey was done on human and animal sera by plaque neutralisation(PN);haemagglutination inhibition and antibody dependent plaque enhancement tests. The viruses tested for are Japanese Encephalitis(JE) Dengue2 WestNile,Chikungunya,Getah,Sindbis,Batai,Sathuperi,Bhanja Lumbo and Snowshoe Hare(SSH).Virus isolation yielded JE from Cx.tritaeniorhynchus,Cx.pseudovishnui,and Cx.gelidus; and Batai from An.vagus,An.peditaeniatus and Cx.fuscocephala. Serpepidemiology has shown
a)Batai,Sathuperi and Bhanja infection in ungulate species,
b)JE activity upto 5300 ft but no evidence for West Nile virus activity.Dengue seropositivity was widespread.
c)the presence of a California serogroup virus/es (Lumbo and SSH PN seropositivity) in the North Central province as shown by antibodies in humans and animals. This is the first evidence for the activity of California serogroup viruses in South/South East Asia.

R35.12

OLIGONUCLEOTIDE FINGERPRINTING OF CHIKUNGUNYA VIRUS STRAINS.

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Extensive febrile epidemics due to chikungunya virus have been reported from different cities in India viz. Calcutta (1963), Madras (1964), Nagpur (1965) and Barsi (1973). T1 RNase resistant oligonucleotide fingerprinting was carried out from the RNA of the different strains isolated from different places and compared with the African (Ross) strains of the virus. The similarity ratios (SR) between the African strains and two Barsi strains were about 0.74. The SR with the two Calcutta strains were 0.53 and 0.57, with the Madras strain it was 0.55 and with the Nagpur strain it was 0.49.

The SR with different strains were compared with each other. The two Calcutta strains were virtually identical while there was slight difference between the two Barsi strains. The Nagpur strain stood apart from the other strains as the SR values were less than 0.5 with all the strains.

R35.13

SUSCEPTIBILITY AND TRANSMISSIBILITY OF DIFFERENT GEOGRAPHICAL STRAINS OF AEDES AEGYPTI TO CHIKUNGUNYA VIRUS.

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Aedes aegypti collected from different Indian cities viz. Pune, Panvel, Aurangabad (Maharashtra State), Delhi and Pondicherry, and a strain maintained in the laboratory for more than 20 years were tested for the susceptibility and ability to transmit the Calcutta strain of chikungunya virus. The mosquito strain from Aurangabad was significantly more susceptible to infection by feeding through membrane as compared with the colony and the Poona strains. Virus was also transmitted by the Aurangabad strain more efficiently as compared with the other strains. The susceptibility and transmission experiments were also carried out by feeding the different mosquito strains on viraemic chickens. The results were concordant with the earlier experiments. A second infective blood meal increased the percentage of infective mosquitoes significantly. The epidemiological implications of the results will be discussed.

R35.14

CRIMEAN-CONGO HAEMORRHAGIC FEVER IN WEST AFRICA

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Following the diagnosis in 1983 of two cases of Crimean-Congo haemorrhagic fever (CCHF) in Mauritania and Burkina Faso, an epidemiological survey was undertaken to determine the distribution of the virus in West-Africa. High levels of CCHF virus circulation were found in southern Mauritania and northern Senegal. The distribution of the virus coincides closely with the known distribution of **Hyalomma** spp ticks. In the southern Mauritania CCHF virus was isolated from 13/58 pools of **Hy.marginatum rufipes** and 1/23 pools of **Hy.impeltatum**. In contrast, no virus was isolated from 208 pools of **Hy.dromedarii**. In the Senegal focus (north and along the Senegal river) CCHF virus was isolated from 30/232 pools of **Hy.marginatum rufipes** and 2/15 **Hy.truncatum**; in western Senegal, the virus was also isolated from **Amblyomma variegatum** (2/88) and **Boophilus geigyi** (2/35).
Antibody to CCHF virus in domestic ungulates was demonstrated by immunofluorescence antibody assay in all major cattle farming areas, and was highly prevalent in southern Mauritania and along the Senegal river. Antibodies were also present in 43/88 camels tested.
A serological survey performed among sheepherds living in the southern Mauritania showed that 8.5% (18/181) had antibodies against CCHF. In contrast, a low prevalence (0.5%) of CCHF antibodies was detected in rural areas of western and southern Senegal (8/1171).
Our data are discussed in relation to information on the current distribution of **Hyalomma** spp in Africa and reported cases of CCHF.

R36.1

PURIFICATION AND CHARACTERIZATION OF A NOVEL SINGLE-STRANDED DNA VIRUS ASSOCIATED WITH SUBTERRANEAN CLOVER STUNT DISEASE IN AUSTRALIA. P.W.G. CHU and K. Helms.
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Subterranean clover stunt(SCS) is an aphid-borne virus-like disease which affects a number of economically important legume crops in Australia. Studies over the last 30 years have failed to identify the causal agent. We have now isolated a novel virus-like particle, 17-19 nm in diameter from SCS infected subterranean clover and peas. The structure and genetic organization of the SCSV suggest that it is representative of a new group of plant DNA viruses.

SCSV are isometric particles sedimenting as a single component with buoyant density of 1.24 in Cs_2SO_4 and 1.34 in CsCl. The A260/A280 is ca. 1.35, indicating a nucleic acid content of 16%-17%. Molecular calculations suggest that the SCSV particle has a T=1 capsid containing 60 polypeptide subunits each with Mr of 19,000 daltons. Nucleic acid analysis including restriction mapping of dscDNAs and preliminary sequence data show that the virus has a divided genome of 3-4 circular, ssDNA molecules of 850-880 nucleotides each. Each DNA is encapsidated in a separate particle. cDNA of SCSV has been cloned and sequence comparisons will determine its relationship with other small DNA viruses.

Both nucleic acid and immunological diagnostic probes have also been developed and used to identify SCS-infected plant from the field.

R36.2

COMPARISON BETWEEN NUCLEOTIDE SEQUENCE OF ABUTILON MOSAIC VIRUS AND OTHER GEMINIVIRUSES BY COMPUTER ANALYSIS
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The Abutilon Mosaic Virus (AbMV) is a geminivirus with a single-stranded DNA. In infected plants (Abutilon sellovianum) a closed-circular double-stranded DNA occurs as a replicative intermediate. This replicative form was isolated from infected plants and cloned in a M13 vector. The cloned DNA was sequenced using the dideoxy-sequencing method.

Partially determined nucleotide sequences of AbMV have more homologies to the whitefly-transmitted geminiviruses Cassava Latent Virus (CLV), Tomato Golden Mosaic Virus (TGMV) and Bean Golden Mosaic Virus (BGMV) than to the leafhopper-transmitted geminiviruses. From those results can be concluded that AbMV is closely related to these whitefly-transmitted geminviruses.

R36.3

WITHDRAWN

R36.4

GEMINIVIRUS DNA IS PRESENT IN PLASTIDS
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The characteristic leaf mosaic of infected Abutilon sellovianum is caused by abutilon mosaic virus (AbMV), a geminivirus with single-stranded (ss) DNA. Although pathological symptoms can clearly be observed in the plastids the virus particles have never been detected in these organelles. Unbroken plastids were isolated from AbMV infected and uninfected plants in order to determine the localization of the viral genome.

As judged by the binding of the fluorochrome DAPI plastids of infected plants showed a larger DNA content than plastids of uninfected plants. Furthermore their thylacoidsystem was degenerated and substituted by amorphous electron-dense material. Southern blot hybridization experiments indicated that the viral ssDNA of AbMV was present in plastids of infected plants. Control plants gave no hybridization signals. DNase treatment of isolated plastids excluded an unspecific adsorption of viral DNA to the plastids. This is the first evidence for the presence of viral DNA in plastids.

R36.5

WHITEFLY-TRANSMITTED GEMINIVIRUSES FROM INDIA.
D. Robinson, I. Roberts, B. Harrison. Scottish Crop Research Institute, Invergowrie, Dundee, U.K. and V. Muniyappa. University of Agricultural Sciences, Bangalore, India.

The causal agents of several whitefly-transmitted diseases in India have been identified as geminiviruses, on the basis of reactions in immunosorbent electron microscopy with antisera to other geminiviruses and/or in spot hybridization tests with a probe for DNA-1 of African cassava mosaic virus (ACMV). Although none of the agents is transmissible by manual inoculation, investigation of their host ranges by transmission with the vector Bemisia tabaci has made it possible to distinguish several viruses. A virus causing leaf curl in tobacco (TLCV) and tomato may be similar to isolates of tobacco leaf curl and tomato leaf curl viruses from elsewhere in Asia and Africa. Horsegram yellow mosaic virus (HGYMV) infects a range of legumes including mungbean, and has a similar host range to mungbean yellow mosaic virus (MYMV) reported from Thailand. However, whereas MYMV reacted strongly with ACMV DNA-2 probes, as predicted from a comparison of their DNA sequences, HGYMV reacted weakly or not at all. This suggests that HGYMV and MYMV may be distinct viruses. Geminiviruses causing yellow mosaic in Dolichos, yellow vein mosaic in bhendi and yellow vein mosaic in the weed Croton bonplandianus differ from each other and from HGYMV, MYMV and TLCV.

R36.6

DNA POLYMERASES IN A CHLORELLA-LIKE GREEN ALGA INFECTED WITH THE VIRUS PBCV-1. J. Rohozinski and J. L. Van Etten. Department of Plant Pathology, University of Nebraska, Lincoln, Nebraska, U.S.A.

PBCV-1 is a large (ca. 190 nm in diameter), polyhedral, plaque forming virus which replicates in certain eukaryotic, unicellular Chlorella-like green algae. The virus contains a large dsDNA genome of 333 kbp, at least 50 structural proteins and a lipid component. PBCV-1 infection is synchronous and progeny viruses first appear 3 to 4 hr after infection. By 6 to 8 hr virus release is complete. The synchrony of infection makes the PBCV-1-Chlorella system a useful model for studying DNA replication, gene expression and regulation in a photosynthetic eukaryotic organism. We have initiated studies on the enzymes involved in virus DNA replication. Inhibitor studies indicate that PBCV-1 DNA synthesis requires either the synthesis of a complete virus encoded DNA polymerase(s) or the synthesis of an essential protein(s) which alters the properties of the host DNA polymerase(s). Crude extracts from uninfected and PBCV-1 infected cells were assayed for DNA polymerase activity. Three forms of DNA polymerase activity were identified in both healthy and virus infected cells. These polymerases can be distinguished from one another by differences in response to inhibitors, conditions required for optimum activity and ability to withstand freezing and thawing.

R36.7

PEANUT CHLOROTIC LEAF STREAK VIRUS, A CAULIMOVIRUS INFECTING PEANUT (ARACHIS HYPOGAEA) IN INDIA.

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Chlorotic streaks along leaflet veins, and stunting of peanuts were shown to be caused by a caulimovirus. The virus has a wide host range in the families Amaranthaceae, Chanopodiaceae, Cruciferae, Leguminosae and Solanaceae. Employing Nicotiana rustica infected tissue, the virus was purified by chloroform treatment, high speed pelleting and gradient centrifugation in potassium tartarate and cesium chloride solutions. Virus particles in leafdip and purified preparations were spherical and of 52 + 3 nm diameter. Cytoplasmic inclusion bodies characteristic of caulimoviruses, with scattered virus particles embedded in the matrix, were observed. Nucleic acid extracted with phenol and chloroform was digested with EN'ase. In 1.0% agarose gels DNA formed two bands of 8.3 and 7.0 kbp. The virus contained three polypeptides of 62,000, 37,000 and 35,000 daltons. This is the first report of a caulimovirus from the Indian subcontinent.

R36.8

THE ORGANISATION AND EXPRESSION OF A GEMINIVIRUS, SEROLOGICALLY RELATED TO MAIZE STREAK VIRUS, FROM DIGITARIA SANGUINALIS. J. Donson, G.P. Accotto*, M.I. Boulton, H. Gunn, P.G. Markham, P.M. Mullineaux, C. Woolston and J.W. Davies, Department of Virus Research, John Innes Institute, Colney Lane, Norwich NR4 7UH, U.K. and *Instituto di Fitovirologia Applicata, Torino, Italy.

The encapsidated single-strand circular DNA of a geminivirus (DSV), isolated from Digitaria sanguinalis, has been sequenced. The data obtained could be accommodated into one DNA circle of 2701 nucleotides. Comparisons of primary sequences and potential amino acid sequences with other members of the geminivirus group will be described. Virion DNA from DSV infected plants also revealed a population of small DNA molecules similar to those found associated with maize streak virus (MSV) preparations. The possible role of conserved sequences in and around these putative primer molecules will be discussed.

In addition an infectious clone of DSV has been sequenced. Comparative experiments, with MSV involving insect transmission of these viruses and the generation of RF II molecules will be described.

R36.9

GENE EXPRESSION FROM THE COMPLEMENTARY SENSE DNA OF THE MAIZE STREAK GENOME. G.P.Harrison, K.J.Kunert, P.M.Mullineaux, M.I.Boulton and J.W.Davies. John Innes Institute. Colney Lane, NORWICH NR4 7UH. United Kingdom.

Transcription from maize streak virus has been demonstrated to be bidirectional (1). Despite being present in very low abundance further complementary (-) sense transcripts have been detected and mapping data for these transcripts will be presented. The anomalous position of the 5' end of the 1.2kb transcript on the complementary sense has been clarified.
Antibodies raised against a peptide encoded by the complementary sense of the genome were used to attempt to detect a polypeptide in in vitro translations of RNA prepared from infected tissue.
The RNA tail on the 5' end of the primer-like molecule (2) has been detected in RNA prepared from infected maize tissue. Data will be presented on the comparison of the RNA moiety of the DNA primer prepared from infected tissue and that isolated from the virus.

(1). Morris-Krsinich B.A.M., et al (1985). Nucl. Acids Res. ,13, 7237-7256.

(2). Donson, J., Morris-Krsinich, B.A.M., Mullineaux P.M., Boulton, M.I. and Davies, J.W. (1984) EMBO J, 3069-3073.

R36.10

THE DETECTION OF NON-STRUCTURAL POLYPEPTIDES ENCODED BY THE MAIZE STREAK VIRUS GENOME. P.M. Mullineaux, R. van der Vlugt, K.J. Kunert, P. Bowyer, G. Harrison and J.W. Davies. John Innes Institute, Colney Lane, Norwich, NR4 7UH, United Kingdom.

Transcript mapping data suggest that at least 4 polypeptides are coded for by the MSV genome. Of these, the virion (+) sense encoded 26969 open reading frame (ORF) codes for the coat protein of MSV.

Restriction fragments from cloned MSV DNA covering the other 3 ORFs have been fused to the 3' end of the lacZ gene so that a B-galactosidase-viral hybrid protein could be synthesized and used to raise antisera. Using one of these antisera a viral specific 11,000 Mr protein has been detected from both in vitro translation reactions and from infected leaf tissue. The 11,000 Mr polypeptide is the product of the 10906 virion (+) sense ORF located 5' to the coat protein ORF. The protein has not been detected in virus particles. Comparison with equivalent ORFs in other geminiviruses has revealed homologies with only those which infect the Graminae. The implications of the existence of this 11,000 Mr protein for the expression of the virion (+) sense part of the MSV genome will be discussed.

Data will be presented on the use of similar techniques to detect polypeptides coded by the complementary (-) sense encoded ORFs.

R36.11

THE REPLICATION OF MAIZE STREAK VIRUS. W.R. McLauchlan, M.I. Boulton, M.S. Marks, P.D. Bowyer, P.M. Mullineaux, P.G. Markham and J.W. Davies, Department of Virus Research, John Innes Institute, Colney Lane, Norwich NR4 7UH, U.K.

The production of maize plants systemically infected with cloned maize streak virus (MSV) using "agroinfection", (Agrobacterium mediated virus infection), has allowed replication studies on this geminivirus which, until recently, has been mechanically non-transmissible.

This study has shown that a dimer composed of a tandem repeated monomer of MSV DNA cloned into the T-DNA of the binary vector Bin19 is infectious when mobilised into Agrobacterium. Results that will be presented include, the infectivity of monomers, the effect on infectivity of the interaction between different Ti plasmids and Agrobacterium chromosomal backgrounds and host range studies within the Graminae, including major crop species.

Virus replication studies have also been carried out using maize cell cultures. Co-cultivation, P.E.G. inoculation and electroporation have been employed to introduce a variety of MSV constructs into maize cells including antibiotic resistance genes to facilitate the selection of transformed calli.

R36.12

HOST-SPECIFIC BY-PASS OF THE POLYADENYLATION (POLY-A) SIGNAL IN CAULIFLOWER MOSAIC VIRUS (CaMV). H. Sanfacon, J.-M. Bonneville, J. Fuetterer, B. Pisan, T. Hohn. Friedrich-Miescher Institut, Basel, Switzerland

The 35S RNA of the plant DNA virus, CaMV, is used as the template for reverse transcription. It spans the entire genome with a terminal redundancy. The polyA site, located 180 bp after the transcription start is therefore not recognized during the first cycle of transcription of the 35S RNA, but efficiently at the second. The template for such a transcript can be either circular, or linear with a terminal redundancy (provirus). We are studying this phenomenon, common to all retroid elements, using a mini-provirus introduced into plant protoplasts by electroporation. All the viral functions have here been replaced by a single CAT reporter gene. CAT expression from this construct is clearly higher in protoplasts from a CaMV host than a non-host plant. Destruction of the upstream polyA site by deletion or point mutation does not affect expression in the host cells. By contrast, in the non-host cells, these mutations enhance the reporter activity. We propose the involvment of a host factor in the by-pass of the polyA signal.

R36.13

INFECTIVITY OF CLONED TANDEM DIMERS OF BEAN GOLDEN MOSAIC VIRUS DNA. M.Ikegami, T.Morinaga, T.Arai, K.Miura. NODAI Res. Inst., Tokyo Univ. of Agricul., Centl. Res. Lab., Teijin Ltd., Dept. of Applied Chem., Univ. of Tokyo, Tokyo, JAPAN

Cloned tandem dimers of each component of bean golden mosaic virus (BGMV) dsDNA was constructed. The mixture of such cloned tandem dimers infects Phaseolus vulgaris "Top crop" plants and elicits symptoms identical to those obtained by native BGMV or its ssDNA , which consists of two components. In this case, yellow mosaic symptoms are accompanied by virus production. Progeny virus isolated from plants infected with such cloned DNAs was indistinguishable from native BGMV on the basis of its particle morphology, serological identity and its circular ssDNA genome. Southern blot analysis of DNA extracted from cells infected with such cloned DNAs revealed the presence of intracellular virus specific ss and dsDNA species as detected in cells infected with the native virus. It is likely that the cloned tandem dimers of BGMV DNA from which the foreign sequence (plasmid) is eliminated by recombination may be efficiently replicated and expressed in plant cells.

R36.14

ABSTRACT WITHDRAWN

R36.15

THE NUCLEOTIDE SEQUENCE OF CLONED CHLORIS STRIATE MOSAIC VIRUS. M.T. Andersen and B.A.M. Morris-Krsinich. Plant Diseases Division, DSIR, Auckland, New Zealand.

Chloris striate mosaic virus (CSMV) is a geminivirus containing single-stranded DNA of 2800 bases. Double-stranded closed circular DNA the putative replicative form was isolated from infected plants and cloned. The complete nucleotide sequence of several clones has been determined. Information relating to the virus sequence will be presented. In addition RNA transcripts mapped to the virus genome will be shown.

R36.16

SPLICING OF AN INTERVENING SEQUENCE FROM HYBRID CAULIFLOWER MOSAIC VIRAL RNA

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Friedrich Miescher-Institut, Basel, Switzerland
*Plant Protection Institute, Hungarian Academy of Sciences Budapest, Hungary.

A DNA sequence encompassing intervening sequence 2 of the soybean leghemoglobin gene was inserted into the cauliflower mosaic virus (CaMV) genome. Upon passage through host plants progeny virus slowly accumulated that had lost the intron, apparently by exact splicing of the RNA form of the genome at the exon/intron borders. These findings suggest that reverse transcription from (spliced) RNA to DNA is a normal step in CaMV replication. S1 nuclease mapping revealed a mixed population of unspliced and spliced viral RNAs. The inefficiency of the splicing process is discussed.

R36.17

THE DNA SEQUENCE OF WDV-C, A WHEAT DWARF VIRUS ISOLATE FROM CZECHOSLOVAKIA.

U. Commandeur, V. Matzeit and B. Gronenborn
Max-Planck-Institut für Züchtungsforschung,
D-5000 Köln 30, FRG.

In addition to the DNA sequence of a wheat dwarf virus isolate from Sweden (WDV-S) we have determined the complete sequence of a WDV isolate from Czechoslovakia (WDV-C).
The comparison of the two DNA sequences reveals a very similar genome organisation between WDV-S and WDV-C. The single-stranded genome of WDV-C has a length of 2750 bases, one longer than the one of WDV-S.
40 base changes result in only a few aminoacid changes concerning the proteins encoded by the open reading frames I - IV. However, most of the base changes in open reading frame V which has minus-strand polarity would lead to drastic aminoacid changes in a potential orf V protein. We therefore assume that this orf occurs fortuitously on the DNA sequence.
One of the WDV-C isolates has a duplication of 172 bases in the large intergenic region. It maps within the stemloop structure common to all geminiviruses. The implications of this duplication on the mode of geminivirus replication will be discussed.

R36.18

TRANSCRIPT MAPPING AND EXPRESSION CHARACTERISTICS OF WHEAT DWARF VIRUS: THE PLUS-STRAND TRANSCRIPTION UNIT. H.-J. Schalk, V. Matzeit, M. Kammann and B. Gronenborn

Max-Planck-Institut für Züchtungsforschung,
D-5000 Köln 30, FRG.

The genome of wheat dwarf virus (WDV) is transcribed bidirectionally. A single abundant WDV-specific transcript of plus-strand polarity is present in infected wheat tissue. It has a length of 1.1kb and spans open reading frames with a coding capacity for an 11kD and a 29.4kD protein. The latter is the capsid protein gene.
The 5' end of this transcript has been mapped 7 bases upstream of the 11kD orf, it terminates about 20 bases after the capsid protein gene.
Gene fusions of the ATG codon of the 11kD orf and the 29.4kD orf to a neomycin phosphotransferase reporter gene reveal that both orfs are translated into protein. The existence of only one transcript of plus-strand polarity suggests that it is a dicistronic mRNA whose expression is post-transcriptionally regulated.
These results are in contrast to the transcription data published on Maize streak virus (MSV).

R36.19

TRANSCRIPT MAPPING AND EXPRESSION CHARACTERISTICS OF WHEAT DWARF VIRUS: MESSENGER RNA OF MINUS-STRAND POLARITY. H.-J.Schalk, V. Matzeit and B. Gronenborn
Max-Planck-Institut für Züchtungsforschung, D-5000 Köln 30, FRG.

The genome of wheat dwarf virus (WDV) is transcribed bidirectionally. Three open reading frames of minus-strand polarity are found on the WDV genome. In contrast to the plus-strand transcription unit transcripts of minus-strand polarity are of very low abundance.
The 5' end of a transcript with minus-strand polarity has been precisely located in front of the open reading frame with the coding potential for a 30kD protein. Probes specific for the second open reading frame (17kD) light up the same transcript as do probes for the 30kD orf.
The frame of the potential 17kD protein does not begin with an ATG codon. A comparative alignment of the 30kD and the 17kD potential proteins of the monocot geminiviruses to a single contiguous orf for a 40kD protein of the dicot geminiviruses reveals a high degree of homology.
Frameshift suppression or RNA-splicing might lead to the expression of a single protein encoded by these two open reading frames of WDV. Evidence for the latter will be presented.

R37.1

ACTUAL INFORMATION STATUS ON NEUROVIRULENCE MARKERS OF EQUINE HERPESVIRUS 1 (EHV 1). F. Bürki[1], M. Engels[2], and N. Nowotny[1].
1) Institute of Virology, Vet. University, Vienna/Austria
2) Institute of Virology, Vet. Faculty, University, Zürich/Switzerland.

Neurological disorders get observed with rising frequency in horses infected with EHV 1. Restriction and protein patterns of a neurovirulent isolate from Lipizzan horses and 5 internationally known reference strains of subtype 1 or 2, respectively, were compared. The Lipizzan isolate as well as a British ataxia-case isolate were classified as typical subtypes 1, but both failed to confirm the genomic neurovirulence marker suggested by Studdert and Fitzpatrick in 1984. Contrary to Chowdhury et al. (1986), Nowotny and Burtscher (1987) succeeded to demonstrate neuropathogenicity for suckling mice of the EHV 1 strain which had paralyzed the Lipizzan horses.

It appears feasible to expect more substantial parameters than presently available for neuropathogenicity of EHV 1 subtypes 1 by subjecting their genomes to additional test procedures. Very recently, Engels et al. (Virus Res. 6, 57, 1986/87) succeeded to differentiate neuropathogenic bovine herpes (BHV 1) strains from non-neuropathogenic strains by means of restriction site mapping and cross-hybridization.

R37.2

DNA FINGERPRINTING AND PLAQUE MORPHOLOGY OF HERPESVIRUSES OF CATTLE AND BUFFALO

K.M. Tham,
Department of Veterinary Pathology & Microbiology. Faculty of Veterinary Medicine and Animal Science, Universiti Pertanian Malaysia, Serdang, Selangor, Malaysia.

Four herpesviruses were isolated from the respiratory and genital tracts of cattle and buffalo. DNAs of these herpesviruses were prepared, digested with restriction enzymes Bam HI and Bgl II and electropherosed in agarose gel. Results showed that the DNAs of the respiratory and genital herpesviruses of cattle had electrophoretic patterns similar to that of the buffalo herpesviruses. However, the respiratory herpesvirus DNAs of the cattle and buffalo had restriction sites that were cleaved distinctly different to that of the genital herpesvirus DNAs. This observation was further supported by the plaque formation of the respiratory and genital herpesviruses of cattle and buffalo in confluent monolayers of bovine embryo kidney cells in which the respiratory herpesviruses of cattle and buffalo yielded smaller plaque sizes compared to that produced by the genital herpesviruses.

R37.3

ASSOCIATION OF CYTOMEGALOVIRUS GENOME WITH AUTOIMMUNE INSULIN-DEPENDENT DIABETES MELLITUS. H. Myong Eun, Chin Y. Pak, Robert G. McArthur and Ji-Won Yoon. Department of Microbiology and Infectious Diseases, Faculty of Medicine, University of Calgary, Calgary, Alberta, Canada.

It was previously reported that a child with congenital cytomegalovirus (CMV) infection developed insulin-dependent diabetes mellitus (IDDM). The present investigation was initiated to find whether the presence of CMV genome is associated with autoimmune IDDM. DNAs extracted from the cultured lymphocytes obtained from 24 IDDM patients or 19 normal controls were dot-blotted on a nylon membrane and hybridized with [^{32}P]-labelled CMV (AD169)-specific probe (1.85 kilobase). About 21% of the recent-onset IDDM patients were found to have CMV genome in their lymphocytes, while none of the controls had such viral genomes in their lymphocytes. To find any correlation between CMV infection and the production of autoantibody, cytotoxic beta cell surface autoantibody (CBSA) was measured in sera from IDDM patients and normal controls. About 45% of all IDDM patients tested were CBSA-positive while none of the controls had such antibodies. However, 83% of the IDDM patients with CMV genome had CBSA, while 34% of the IDDM patients without CMV genome had such antibodies. It is concluded that CMV genome in about 18% of all tested IDDM patients are strongly associated with the induction of autoimmune IDDM.

R37.4

MUCOCUTANEOUS HERPES SIMPLEX VIRUS INFECTIONS IN IMMUNOCOMPROMISED JAMAICAN PATIENTS. P. Prabhakar, Department of Microbiology, Faculty of Medical Sciences, University of the West Indies, Kingston 7, Jamaica.

Thirteen immunocompromosed patients at the University Hospital of the West Indies developed mucocutaneous herpes simplex virus (HSV) infections between 1982-85. The mean age of patients was 45 years (range 25-83 yrs). There were 8 females and 5 males. Three patients were on steroid therapy, two on cytotoxic therapy and 4 patients on both. There were 16 HSV infections of which 4 were disseminated, 3 were genital, 4 were perineal, and 4 were visceral (3, proctitis and 1, proctocolitis). Twelve patients had virological confirmation of HSV infections, of which 9 infections were confirmed as HSV-2. Our data shows that HSV infections in the immunocompromised patients is not uncommon; hence the effectiveness of current antiviral agents for the treatment of HSV infections in immunocompromised Jamaican patients should be evaluated.

R37.5

EFFECT OF Ø 6 RNA BACTERIOPHAGE ON HERPESVIRUS INFECTIONS. H.Sandoval-Trujillo†, M.A.Martínez-Rivera & B.L.Barrón-Romero. Departamento de Microbiología, Escuela Nacional de Ciencias Biológicas† I.P.N. México D.F. 11340 México. *Departamento de Sistemas Biológicos, Universidad Autónoma Metropolitana, Unidad Xochimilco. México D.F. 04960 México.

The antiviral activity of purified double stranded Ø 6 RNA was determined "in vitro" with MA-104 and Vero cells, and "in vivo" with female guinea pigs (400-600 g).

Cells grown in microwell dishes were inoculated with different concentrations (25,10,1,0.1,0.01 ug/ml) of Ø 6 RNA; 18 hours later they were infected with 200 $TCID_{50}$ of either HSV-1 or HSV-2. The Ø 6 RNA Protection Dose 50% (PD_{50}) was calculated by the Kärber formula. For MA-104 cells the PD_{50} for HSV-1 was 1.68 ug/ml and for HSV-2, 0.25 ug/ml of Ø 6 RNA. Under these conditions, Vero cells were not protected from the two herpesvirus infections.

Guinea pigs were inoculated by a single vaginal dose of HSV-2. A dose of $2x10^3$ $TCID_{50}$ induced clinically recognizable disease in all the inoculated animals, 3 to 5 days after inoculation. A higher dose ($2x10^5$ $TCID_{50}$), caused severe genital lesions and death in a shorter time. Guinea pigs treated with a single intramuscular dose of 100 ug/Kg of Ø 6 RNA, one hour before HSV-2 vaginal inoculation, showed a lower rate of infection, a mild disease and less mortality. Only 1 out of 4 inoculated animals with $2x10^3$ $TCID_{50}$ HSV-2 and 2 out of 4 inoculated with $2x10^5$ $TCID_{50}$, had clinical infection and died.

R37.6

DEVELOPMENT OF HUMAN CYTOMEGALOVIRUS MAJOR 65K PROTEIN(S) IN INFECTED CELLS.

K. S. Ishida, Xu Bin, S. Ishida, T. Murayama and T. Furukawa Department of Microbiology, Kanazawa Medical University, Uchinada, Ishikawa, JAPAN.

A major structural protein of human cytomegalovirus (HCMV) is a polypeptide with the molecular weight between 64K and 69K, which is located in the virus tegument. The study was undertaken to determine the development and localization of this protein(s) in the infected cells using the monospecific and monoclonal antibodies against a 65K polypeptide. Guinea pig specific antisera to a 65K were prepared by immunization with a 65K polypeptide extracted from SDS-PAGE and monoclonal antibodies were obtained from several institutes. Both antibodies reacted with a 65K by an immunoblotting assay. Antigens detected by immunofluorescence with monospecific antisera first appeared at 24h pi. in cytoplasm and accumulated in the cytoplasmic inclusions (CI) toward late stage of the infection. Non specific staining of the immunoglobulin G receptor (FcR) was ruled out by pretreating this receptor with seronegative sera and also by the anti-complement immunofluorescent staining. Since antigen reacted with the monospecific antisera was localized in CI and a monoclonal antibody reacted with nuclei and cytoplasm, these results suggested the mass of CMV 65K protein(s) contain several antigenic determinants and the antigen may accumulate in CI.

R37.7

MOLECULAR CLONING AND EXPRESSION OF EPSTEIN-BARR VIRUS (EBV) GENES IN E. COLI USING THE EXPRESSION VECTOR λ gt11..... G. Chelkon and A.D. Riggs.....Department of Molecular Biology, Beckman Research Institute, City of Hope National Medical Center, Duarte, California, U.S.A.

Three cosmid clones covering about 70 percent of the EBV-DNA derived from strain M-ABA were used to construct three libraries of EBV-DNA fragments in the expression vector λ gt11. The libraries were screened with a pool of high anti-EBVhuman sera to isolate bacteriophages expressing EBV-encoded proteins. So far, four different recombinant clones have been isolated and mapped to open reading frames of B95-8 EBV-DNA. By the nature of the λgt11 vector, the cloned DNA fragments were expressed in E. coli as β-galactosidase fusion proteins. One fusion protein has been purified from induced lysogens, and rabbits have been immunized. Human antibodies bound and eluted from this fusion protein (160kDa) identified a late EBV-induced protein of 68kDa in the producer cell line B95-8. Studies involving a panel of high anti-EBV positive human sera gave evidence that only a few sera had high antibody titers to this late protein, presumably the product of the BALF2 open reading frame (ORF). Among the other fusion proteins, two represent products of early genes based on the encoding ORF's (BORF2, BLLF2) and adjacent promotors. The production of antibodies to the isolated fusion proteins is in progress to identify and characterize the proteins produced by EBV-DNA carrying cells.

R37.8

INTERACTIONS OF HUMAN CYTOMEGALOVIRUS WITH LEUKOCYTES IN VIVO: ANALYSIS BY IN SITU HYBRIDIZATION. L. Turtinen, N. Saltzman, M. Jordan, A. Haase. Department of Microbiology, University of Minnesota, Minneapolis, MN, USA

Reactivation of human cytomegalovirus(HCMV) from latency occurs in immunosuppressed individuals and infection itself is immunosuppressive. To better understand the basis for this virally induced impairment of immune function, we have analyzed virus-leukocyte interactions by in situ hybridization in 12 viremic patients. We detected viral DNA in the mononuclear cell population predominantly in cells identified as monocytes by their morphology and by labelling the cells with a monocyte specific monoclonal antibody prior to in situ hybridization. Infected cells contained immediate early RNA at frequencies comparable to DNA (10^{-3} to 10^{-5}). By contrast, no viral transcripts were detected in polymorphonuclear cells and viral DNA (found at cell frequencies of 10^{-3} to 10^{-5}) was exclusively cytoplasmic in accord with the interpretation that this cell type harbors HCMV in phagosomes. These findings in vivo continue to suggest that infection of monocytes plays an important part in the immunosuppressive effects of HCMV infection.

R37.9

INDUCTION OF HUMAN CYTOMEGALOVIRUS IMMEDIATE-EARLY GENES BY HEAT SHOCK AND ARSENITE. J.L.N.C. Geelen and J. van der Noordaa. Department of Virology, Faculty of Medicine, University of Amsterdam, Amsterdam, The Netherlands.

Stably transformed cell lines were established by cotransfection of the HSV-TK gene and restriction endonuclease fragments encoding the major immediate-early (IE) genes of human cytomegalovirus (HCMV; strain AD 169). Under normal growth conditions expression is limited to less than 0.1 % of the cells. Upon protein synthesis inhibition a massive increase in CMV-specific transcripts is observed (Boom et al, 1986), which results from a transcriptional activation. The transcriptional activation of the repressed IE CMV genes was analysed by nuclear transcription rates and comparison of the stability of the induced transcripts. In addition the silent CMV genes can be activated by heat shock and arsenite treatment, as documented by immunofluorescence, northern blot analysis and immunoprecipitation of the induced CMV proteins.
The induction by heat shock of the repressed CMV gene is is one of the first examples of a heat shock inducible eukaryotic viral gene, although induction of heat shock proteins by animal viruses has been described. The inducibility of the CMV IE genes in these rat cell lines will be important to elucidate factors which may be involved in latency and reactivation of CMV.

R37.10

ORAL ACYCLOVIR AND HERPES LABIALIS: A RANDOMISED DOUBLE-BLIND, PLACEBO-CONTROLLED STUDY. G.W. Raborn, W.T. McGaw, M. Grace, L.D. Tyrrell, S. Samuels. Department of Stomatology, Faculties of Dentistry and Medicine, University of Alberta, Edmonton, Canada.

Oral acyclovir 200 mg. capsules were tested in a randomized, placebo controlled, double blind study on 210 patients who cultured positive for recurrent herpes labialis. Medication was administered five times per day for five days. One hundred and forty-nine patients were followed over three episodes each on either ACV or placebo. ACV patients fared better during episode one for time to loss of lesion crust ($p<0.05$). The results for episode two favored ACV again for loss of lesion crust and for the area of the lesion measured at day 1 relative to day 5 ($p<0.05$). Episode three also had results favoring ACV when lesion areas were compared on days 1 and 5. Virology data was collected for 210 patients and 514 episodes. A significant antiviral effect was demonstrated by ACV ($p<0.05$) for patients cultured positive on day 1 who remained positive on 5.

R37.11

PREDICTION OF ANTIGENIC EPITOPES IN HERPES SIMPLEX VIRUS GLYCOPROTEIN D. M.J.Redmond, N.C.J. Strynadka, J.M.R. Parker, R.S. Hodges and D.G. Scraba.

Department of Biochemistry, University of Alberta, Edmonton. Canada.

A new predictive algorithm has been used to identify potential linear antigenic sites on herpes simplex virus type-1 glycoprotein D. This analysis was verified by producing rabbit antisera to peptide conjugates, selected from sequences predicted to be on the protein surface, together with control regions of predicted internal sequences. The polyclonal sera produced in response to these peptides was then screened by ELISA for reactivity against peptide conjugates, isolated glycoprotein D and purified HSV virions. We have found that all the peptide sequences selected produced strong immune responses and the predicted surface peptides all reacted well with the isolated glycoprotein. However, reactivity with intact herpes virions indicates that there are definite constraints imposed on the surface exposure of glycoprotein D during the assembly of the viral envelope. In assays of virus neutralization, we have unveiled an antigenic site removed from the N-terminal region which elicits antibodies with potent virus neutralizing activity. This region is also recognized by human sera from HSV positive individuals and should therefore be of interest in terms of synthetic vaccine development.

R37.12

TYPE OF HERPES SIMPLEX VIRUSES ISOLATED FROM FEMALE GENITAL HERPES AND ITS CLINICAL SIGNIFICANCE IN JAPAN. T. Kawana[1], M. Hashido[2]. Department of Obstetrics & Gynecology[1], Department of Maternal and Child Health[2], Faculty of Medicine, University of Tokyo, Tokyo, Japan.

Type of herpes simplex virus (HSV) isolated from female genital and non-genital sites were determined. 35 strains from non-genital sites were typed as HSV-1 except one. Among 332 strains from female genital sites, 149(44.9%) were typed as HSV-1 and remaining 183(55.1%) were typed as HSV-2. Among 14 strains from male genital sites, all but one were typed as HSV-2. 116(65.2%) among 178 patients with primary genital herpes infection were caused by HSV-1. On the other hand, 58(94%) out of 62 recurrent type and 35(90%) of provoked type were caused by HSV-2. Thus, HSV-2 were more closely related to latent infection in genital region. All primary genital herpes in the patients younger than 10 years old and 80% in those older than 50 years old were caused by HSV-1. Prospective study showed that 13(81%) out of 16 HSV-2 primary infection recurred within 6 month, whereas 6(17%) out of 35 HSV-1 primary infection recurred. Thus, it was suggested that primary genital herpes caused by HSV-2 recurred more frequently than HSV-1.

R37.13

CHARACTERIZATION OF THE EPSTEIN-BARR VIRUS CODED THYMIDINE KINASE. E. Littler and J.R. Arrand. Department of Molecular Biology, The Paterson Institute for Cancer Research, Christie Hospital and Holt Radium Institute, Manchester, U.K.

We have recently reported that the BXLF1 open reading frame of Epstein-Barr Virus (EBV) codes for a protein with thymidine kinase (TK) activity. We describe here the characterization of the EBV TK expressed in eukaryotic and prokaryotic systems and by in vitro transcription/translation of plasmid DNAs. The enzyme expressed in these systems has been shown to utilize all four common nucleosides as substrates in the order dT, C, U, A and to use the four nucleotide triphosphates with the following order of preference CTP G, A and UTP. We are particularly interested in the ability of the EBV TK to utilize antiviral nucleoside analogues as substrates, by direct assays we have shown that both acyclovir and cytosine arabinoside are utilized by the EBV TK's, whilst by competitive inhibition studies we have shown that the EBV TK can be inhibited by the antivirals BVDU, DHPG and Acyclovir in that order.

R37.14

IN VITRO REACTIVATION OF LATENT HERPES SIMPLEX VIRUS (HSV) FROM NEURAL AND EXTRANEURAL TISSUES. D.I. Bernstein, J.C. Kappes, Gamble Inst. Med. Res., Cincinnati, Ohio USA.

The events that lead to the reactivation of latent HSV are not well understood. Recent studies by us and others have suggested that demethylation may play a role in HSV reactivation. We evaluated the demethylating agent hexamethylenebisacetamide (hex) for its effect on reactivation of HSV in vitro. Tissues from guinea pigs inoculated intravaginally with HSV-2 50-150 days before sacrifice were divided into 4 explant cultures, 2 of which received hex. HSV was recovered earlier and from a higher percent of dorsal root ganglia (DRG) explant cultures treated with drug than from cultures not treated. For example, by day 7, HSV was recovered from 35/84 (42%) explant DRG cultures treated with hex compared to 7/84 (8%) cultures without drug ($p < .0001$).

Hex treatment of vaginal and cervical explant cultures also significantly increased the reactivation of HSV so that by day 14 HSV was recovered from 16/66 (24%) vaginal explant cultures treated with hex compared to 5/66 (8%) of controls ($p < .009$). Since hex had no effect on acute HSV replication this implies that HSV is present in these tissues in the same state of latency as in neural tissue. These studies support the hypothesis that methylation of viral genomes plays a role in viral latency and reactivation in neural and extraneural tissue.

R37.15

ISOLATION OF NOVEL HUMAN LYMPHOTROPIC HERPESVIRUSES. M.L.B. Becker*, S. Engelbrecht*, W.B. Becker*, P. Jacobs**. *Department of MEDICAL VIROLOGY, University of STELLENBOSCH, TYGERBERG, S.A., **Department of HAEMATOLOGY, University of CAPE TOWN, CAPE TOWN, S.A.

Lymphotropic viruses other than retroviruses may be involved in the pathogenesis of leukaemia and AIDS. Novel herpesviruses have so far been successfully isolated from the peripheral blood lymphocytes of a patient with hairy cell leukaemia and another with AIDS.

The viruses could readily be transmitted to cultured cord blood lymphocytes but not to primary monkey kidney epithelial cell or human embryonic fibroblast cultures. Infection produced giant refractile lymphocytes which were not transformed but were lysed within 7-10 days and infection could readily be transmitted in the cell-free supernate of the cultures.

Electronmicroscopy of ultrathin sections of infected lymphocytes revealed numerous intra- and extracellular herpesvirus particles.

Viral DNA extracted from infected cells did not hybridize with specific DNA probes for HVH, VZV, CMV nor Epstein-Barr virus.

The role of these viruses in human disease needs to be elucidated.

R37.16

A COMMON ANTIGENIC DETERMINANT BETWEEN HUMAN CYTOMEGALOVIRUS, ADENOVIRUS, AND HERPES SIMPLEX TYPE 2 VIRUSES DETECTED BY A MONOCLONAL ANTIBODY. M.A.Wabuke-Bunoti, A.J.Haefs and R. Gehrz. Biomedical Research Center, Children's Hospital, St. Paul, MN 55102. A murine monoclonal antibody 9E10 was generated against virions of human cytomegalovirus strain Towne. This monoclonal antibody immunoprecipitated at least two HCMV (strain Towne) glycoprotein complexes which on reduction yielded several glycoprotein components with varying molecular weights (B.Kari,et.al., J.Virol.1986,60:345-352). When it was tested for immunological reactivity against other viruses, mcAb 9E10 immunoprecipitated the penton base of adenovirus types 2 and 5, and a protein of approximately 150,000 daltons from HSV-2 (tentatively identified as VP5/NC-1). Monoclonal antibody 9E10 neutralized HCMV infectivity in the absence of complement but as expected, it had no neutralizing activity against adenovirus, and HSV-2 viruses. These results suggest that a common structural antigenic epitope is shared by these DNA viruses. Implications of the functional significance of relatedness between the proteins of HCMV, HSV-2 and adenoviruses still remain unknown. A possibility also exists that recognition of unrelated viral proteins by mcAb 9E10 can be a consequence of molecular mimicry.

R37.17

Serosurveillance of Human Immunodeficiency (HIV) infection in North India. U.K. Sharma and P. Seth Department of Microbiology, All India Institute of Medical Sciences, New Delhi. Serosurveillance of HIV infection in North India has been carried out to get an overview of this problem. serum samples from 1722 promiscuous heterosexual men and women, 219 prostitutes, 20 vigilance home inmates, 5 homosexuals, 4 recipients of blood and blood products, 61 blood donors, 164 foreign students, 3 patients with full blown AIDS and 26 normal healthy individuals were tested for the presence of HIV antibodies by ELISA. The serum samples found positive by ELISA were confirmed by Western Blot. Seven students (6 from Kenya, 1 from Malawi) and 3 patients with AIDS were found positive by ELISA as well as WB, whereas none of the samples from indigenous population were found positive. One of the students was bisexual while six students admitted heterosexual relationship withprostitutes in the country of their origin. Two students had generalised lymphadenopathy and the rest were asymptomatic. The patients with AIDS acquired infection outside India. In WB, antibodies were detected to all antigens of HIV (gp 160/120,p-66,p-51-55,gp 41,p-31,p-24 and p-17) except in one patient who had antibodies to gp 160/120 and gp 41 only. The T helper/suppressor ratio in 1 patient with AIDS, 2 students with generalised lymphadenopathy and 2 asymptomatic students varied from 0.29-0.71 and did not show any correlation with the severity of infection.

R37.18

TOPICAL 3% EDOXUDINE CREAM IN THE TREATMENT OF RECURRENT GENITAL HERPES INFECTION. S.L. Sacks 1, J. Portnoy 2, L.D. Tyrrell 3, W.Schiech, III 4, F.Y. Aoki 5, S. Landis 6, J. Gill 7, A.G. Martel 8, J. Singer 9. University of British Columbia, Vancouver, BC 1, Microbiology Laboratory Clinic, Montreal, PQ 2, University of Alberta, Edmonton, Alberta 3, Victoria General Hospital, Halifax, Nova Scotia 4, Health Sciences Centre, Winnipeg, Manitoba 5, McMaster University, Hamilton, Ontario, 6, University of Calgary, Calgary, Alberta 7, Le Centre Hospitalier de l'Université Laval, Quebec, PQ 8, Ortho Pharmaceutical (Canada) Ltd., Don Mills, Ontario 9, Canada.

Topical 3% edoxudine cream (EDU) was studied in two double-blind, randomized trials in the treatment of recurrent genital herpes (GH). 129 patients were evaluated in a patient-initiated (PI) trial, and 187 in an early-treatment (6 h from onset to drug application) clinic-initiated (CI) trial comparing 5 days of 6 times daily treatment with EDU and placebo (P). 68/129 (PI) and 93/187 (CI) received EDU. Women (all lesions) in the PI study healed in 4.74 (EDU) vs 7.12 (P) days ($P = 0.015$), while men healed in 5.55 (EDU) vs 5.61 (P) days ($P = 0.44$). Time to crusting in women (PI) was 4.01 (EDU) vs 5.62 (P) days ($P = 0.035$). The duration of itching was reduced from 7.50 (P) to 4.08 (EDU) days ($P = 0.009$) in women and lesion pain duration in men was 3.33 days (EDU) vs 6.18 days ($P = 0.08$), in those patients who applied treatment within 2 h of prodrome onset. Viral shedding (PI) from presenting lesions in women lasted 3.94 (EDU) vs 4.24 (P) days ($P = 0.24$), and in men lasted 2.83 (EDU) vs 4.67 (P) days ($P = 0.015$). Nonlesional prodromes were more common in EDU-treated men and women, although these differences were not statistically significant. Treatment was slightly delayed, but variability minimized by CI design. During the CI study, virus shedding in women lasted for a mean of 2.28 days (EDU) vs 3.65 (P) days ($P = 0.004$), while virus shedding in men lasted 2.64 (EDU) vs 3.72 (P) days ($P = 0.0006$). Clinical efficacy parameters observed during the CI trial will be presented.

We conclude that topical 3% edoxudine cream effectively reduced the duration of viral shedding from recurrent GH lesions in both sexes, and offered benefit for several clinical parameters when used during the prodromal phase.

R37.19

ELECTRON MICROSCOPIC STUDY OF TS MUTANTS OF HSV-1 AT PERMISSIVE AND NON-PERMISSIVE TEMPERATURES. Jia-ju Zhang, Tao Hun§, Jia-ye Zhou. Shandong Academy of Medical Sciences. Jinan, China.

To study morphogenesis of Ts mutants of HSV-1, monolayer cultures of HeLa cells were infected with Ts mutants (the DNA⁻ 114 strain supplied by Dr.B.Roizman) and harvested at defined times after inoculation at both permissive and non-permissive temperature. The following essential results were recorded:

1. When cultivated in HeLa cells under non-permissive temperature, the DNA⁻ ts 114 strain assembled staw-berry like particles, associate with numerous tiny tubular structures, in the nucli of the cells. The former particles were thought to be defectve virus formed under non-permissive temperature and the latter the sub-units making up the capsid.

2. Under permissive temperature no remarkable changes were observed as compared with normal wild strains.

3. The information obtained from the present study should be helpful in understanding the mechanism of the morphogenesis of herpes virus.

R37.20

INFLUENCE OF HOST AND VIRAL FACTORS ON INITIAL GENITAL HERPES SIMPLEX VIRUS (HSV) INFECTION IN GUINEA PIGS. L.R. Stanberry, M.G. Myers. Children's Hospital Research Foundation, Cincinnati, Ohio, USA.

Initial genital HSV infection in humans and guinea pigs varies from asymptomatic infection to severe skin disease with associated urologic and neurologic symptoms. Because the host and viral factors affecting the severity of disease are largely undefined we explored whether animal strain, animal age or inoculum titer influenced the course of genital herpes. The duration and severity of skin disease and occurrence and duration of urinary symptoms were influenced by age but were similar in inbred (Strain 2) and outbred (Hartley) animals; beyond the threshold necessary for initiation of clinical disease these were not influenced by inoculum titer. Neurologic symptoms and death were more frequent in inbred and in younger animals. The magnitude of inoculum titer affected the proportion of animals with asymptomatic infection (characterized by brief HSV replication in the genital tract without evidence of clinical disease) or clinically apparent infection. While the inoculum required to induce infection was similar, the inoculum necessary to produce either asymptomatic or symptomatic infection differed for inbred and outbred animals. The manifestation of initial genital herpes may be influenced by both host and viral factors.

R37.21

YOUNG CHILDREN AS A SOURCE OF MATERNAL AND CONGENITAL CYTOMEGALOVIRUS INFECTION. R. Pass, S. Stagno, W. Britt, C. Alford. Department of Pediatrics, University of Alabama at Birmingham, Birmingham, USA.

We investigated 7 families in which there was a recent congenital or maternal cytomegalovirus (CMV) infection. Restriction endonuclease (RE) techniques were used to compare DNA of CMV isolates collected from family members. In 5 families there was a congenitally (4) or perinatally (1) infected infant born after a primary maternal infection during pregnancy, and an older child less than 3 years of age who was excreting CMV. In each of these 5 families, CMV strains from the infected newborn and the sibling were identical. In 2 families CMV was isolated from the mother and was identical to isolates from each of the children. Transmission of CMV from young child to mother during pregnancy seems likely based upon the fact that the siblings were excreting CMV, all had attended day nurseries, 3 of 4 fathers tested were seronegative, and primary maternal infection occurred during the most recent pregnancy as well as the RE results. In 2 additional families acquisition of CMV by a child in a day care center was followed by maternal seroconversion and excretion of CMV identical by RE analysis to that of the child. These results not only strengthen evidence for transmission of CMV from child to mother, but indicate that maternal infections acquired from a child can be transmitted to the fetus.

R37.22

EFFECTS OF INHIBITORS OF GLYCOPROTEIN PROCESSING ON THE ASSEMBLY, RELEASE AND INFECTIVITY OF HUMAN CYTOMEGALOVIRUS. W. J. Britt, L. G. Vugler. Department of Pediatrics, University of Alabama at Birmingham, Birmingham, USA.

Inhibitors of glycoprotein processing were used to study the assembly and release of human cytomegalovirus (HCMV). Inhibition of N-linked glycosylation with tunicamycin (TM) markedly reduced the release of infectious particles, whereas the assembly and release of non-infectious, DNA containing particles was less affected. Likewise, monensin (MN) inhibited the production of infectious extracellular particles by some 99%, yet only reduced the release of noninfectious, viral DNA containing particles by 10%. Because both compounds (TM, MN) prevented processing of HCMV envelope glycoproteins, these results suggested that although infectivity was dependent on complete processing of envelope glycoproteins, assembly and release of particles continued in the absence of mature glycoproteins. Additional support of these results was obtained with the use of swainonine and deoxynojirimycin which inhibit golgi associated glycoprotein processing. Together, these results suggested that HCMV assembly and release can occur through at least two distinct intracellular pathways.

R37.23

RE-EVALUATION OF RECEPTOR-MEDIATED ENDOCYTOSIS AND MATURATION PROCESSES OF PSEUDORABIES VIRUS. H.Hampl, D.Sawitzky, H.Zeichhardt & K.-O.Habermehl. Institute for Clinical and Experimental Virology, Free University of Berlin, Germany.

Monoclonal antibodies directed against the envelope glycoprotein gIII neutralize pseudorabies virus (PrV) infectivity by inhibition of virusadsorption to permissive rabbit kidney cells. As the neutralization induced by monoclonal antibodies reduces the infectivity up to 3 log 10, this reaction of gIII with the cell membrane plays an important role in infecting the cell. Electron microscopic investigations show that in our system endocytosis is the mechanism of virus entry. Shortly after infection the infectious PrV-particles can be seen in the cytoplasm of the infected cell with an intact envelope and the membrane of the vacuole surrounding the particle. Starting at 20 min p.i., virions with intact envelope are pooled in bigger vacuoles containing up to 6 or more virus particles. After 1 h p.i., most of the virus is in big vacuoles and the deenvelopment starts within the vacuoles. At 2 h p.i., single PrV-capsids in the cytoplasm are close to the nuclear membrane often at nuclear pores. Those capsids are mainly without DNA. At that time no newly synthesized capsids are detectible in the nucleus, whereas 3 h p.i. areas of capsid formation and newly produced capsids are visible.

R37.24

MOLLUSCUM CONTAGIOSUM VENEREUM, A SIGNIFICANT STD? J. Dennis, L.S. Oshiro, State Department of Health Services, Berkeley, California, USA.

Molluscum contagiosum virus (MCV) infection is being noted increasingly as a sexually transmitted disease (STD) in young adults presenting at venereal disease, dermatology, and obstetrics and gynecology clinics. The lesions in moist genital areas may become inflamed or ulcerated, and may be confused with lesions produced by herpes simplex virus (HSV). MCV cannot be serially propagated in cell culture, but it undergoes an abortive infection with the production of a characteristic cytopathic effect (CPE). Because MCV CPE disappears with serial passage, it might be considered a toxic effect and such specimens reported negative for a viral agent. MCV CPE occasionally can be mistaken for that produced by HSV, and this can lead to a false diagnosis of HSV infection if specific immunological methods are not used. During a 37 month period, 306 of 688 specimens cultured for HSV produced a CPE. Two hundred sixty-nine were positive for HSV by immunofluorescence staining and 37 were negative. Electron microscopy (EM) on the original specimens or first passage cell culture material confirmed the presence of MCV in 34 specimens. Laboratories involved in HSV isolation should be aware that MCV may be present in lesion specimens that produce a CPE which cannot be specifically identified as that of HSV and cannot be serially passaged. Until MCV immuno- or probe diagnostic reagents become available, the original specimen, or first passage cell culture material, should be submitted to a reference laboratory for EM examination.

R37.25

DELETION OF pg63 OR gI FROM PSEUDORABIES VIRUS DOES NOT DETECTABLY AFFECT THEIR GROWTH CHARACTERISTICS. T. Mettenleiter, C. Schreurs, T. Ben-Porat, Department of Microbiology, Vanderbilt University School of Medicine, Nashville, TN 37232.

Several vaccine strains of PrV, including the Bartha strain, have a deletion in the S component of their genome encoding glycoproteins gI and gp63 (Lomniczi et al., 1984; Mettenleiter et al., 1985; Petrovskis et al., 1986). The Bartha strain grows well in rabbit kidney (RK) cells, but is released poorly from these cells (Ben-Porat et al., 1986); after an intact S component has been restored to it, it is released well. To determine whether the deletion of either gI or gp63 (or both) from the wild type virus genome will affect the release of virions from RK cells, we have constructed mutants of wild type PrV with defects in these genes. To this end, either cotransfection of cells with wild type DNA and a fragment of PrV DNA containing the gene into which a deletion has been introduced or with wild type DNA and a fragment of PrV containing the gene into which sequences from M13 had been introduced, was used. Both methods yielded the desired mutants. All the mutants behaved like wild type virus; i.e., were released efficiently from RK cells. We conclude that the absence of gI or gp63 does not affect release of standard virus from RK cells. The expression of these genes is, however, important to the release of the Bartha strain. Therefore, these gene products must act in conjunction with some other (unknown) function which appears to be defective in the Bartha strain.

R37.26

ABSTRACT WITHDRAWN

R37.27

PROTECTIVE EFFECT OF AND IMMUNE RESPONSE TO A FORMALIZED HSV-1 SUBUNIT VACCINE IN GUINEA PIGS. R.J. Philpotts, P. Ridgeway, M. Welch, A.C. Walkland, J. Melling, Vaccine Research & Production Laboratory, PHLS Centre for Applied Microbiology & Research, Porton Down, Salisbury, Wiltshire, England.

Herpes simplex viruses (HSV), which cause mucocutaneous infections of man, have the ability to remain latent in dorsal root ganglia, and reactivation of latent virus can lead to a recrudescence of lesions. The virus-specific immune response is involved in the termination of both primary and recrudescent infections, and in the control of recrudescence. Therefore vaccination may be a feasible method of controlling HSV disease.

In the present study, a guinea pig model of HSV-2 infection was used to provide a reproducible potency test for a formalized, HSV-1 subunit vaccine, and the virus-specific glycoprotein antigen content of the vaccine preparations was estimated using an ELISA technique. The humoral immune response of guinea pigs to vaccination was compared to that produced by live virus challenge using a variety of antibody functional and binding assays.

R37.28

EQUINE HERPES VIRUS 1; GENE ORGANIZATION IN THE LONG UNIQUE REGION.
G. Robertson, G. Hudson, N. Scott and J.M. Whalley
School of Biological Sciences, Macquarie University, Sydney Australia.

The gene organization in three separate regions of the long segment of EHV-1 DNA has been analysed to assign specific genes and compare their location and sequence with analogous genes of other herpes viruses.

The EHV-1 gene equivalent to that of HSV-1 gB, a significant glycoprotein in eliciting an anti-viral immune response, was initially identified by hybridization with a HSV-1 probe. The 4.2 Kb DNA sequence of this region (map units 0.40 to 0.43) showed extensive homology to the HSV-1 gB and varicella zoster virus gpII genes, and the predicted protein had significant similarities on hydropathic plots. The region of EHV-1 coding for thymidine kinase was located between map units 0.46 and 0.48 by DNA-mediated transformation of LMTK$^-$ cells, although the DNA sequence of this region showed little homology with the Tk gene of HSV or VZV. The genes for the EHV-1 major capsid protein and EHV-1 equivalents of VZV genes 40 and 41 were located between map units 0.54 and 0.56, and had high homologies with HSV and VZV. This information provides a basis for studying several important genes of EHV-1 and comparing the gene organization in the long unique region of herpes viruses.

R37.29

PURIFICATION AND CHARACTERIZATION OF A 30,000-DALTON GLYCOPROTEIN OF HSV-2. Qin Ke-feng, Wang Mei-xian, Jiang Shao-zun, Ma Wen-yu, Dept. Microbiology, 4th Military Medical College, Xian, Shaanxi, China.

A 30,000-dalton glycoprotein(g30K) of HSV-2 was purified by affinity chromatography. The McAb CH-A9 was used in these studies; CH-A9 has been shown by indirect ELISA and IF on fixed and unfixed HSV-infected BHK cells to be specific for a HSV-2 glycoprotein. A cell membranous extract prepared from either HSV-1 or HSV-2 infected or normal BHK cells was loaded onto an immunoadsorbent column consisting of the McAb CH-A9 linked to Sepharose 4B and then the column was eluted with 3M KSCN. Only on the elution curve of the cell membranous extract prepared from HSV-2 infected BHK cells a peak could be observed.

Evidence is presented which shows that the g30K has properties distinct from those assigned to any other HSV glycoprotein. Firstly, its molecular weight (30,000 dalton) determined by SDS-PAGE is lower than other HSV glycoproteins. Secondly, the glycoprotein is HSV-2 type specific shown in ELISA tests. Finally, this glycoprotein can induce HSV-2 type specific neutralizing antibody in BALB/c mice.

R37.30

HUMAN HERPESVIRUS - TYPE 6 (HHV-6) PRELIMINARY CHARACTERIZATION OF A NEW HUMAN VIRUS. P. Feorino, P. Pellett, J. Stewart, C. Goldsmith, K. Sanderlin, C. Lopez, Centers for Disease Control, Atlanta, Georgia, U.S.A.

During routine culture of human mononuclear cells, a cytopathogenic agent was found which suppressed replication of HIV. Such an agent has now been found in lymphocyte cultures from nine individuals: 8 were HIV seropositive and one was HIV seronegative. Electron microscopic (EM) investigations revealed evidence of a herpesvirus found in blasting lymphocytes and in some cells which appeared to be macrophages. The proportion of cells replicating virus varied from <1% to about 50%. Nucleocapsids were found within the nucleus of infected cells and enveloped virus was detected budding into the golgi in the cytoplasm. Inoculation of PHA and IL-2 stimulated cord blood lymphocytes with this agent was followed by peak virus replication 4-8 days later. Some human sera clearly reacted with only CMV, EBV, HSV-1 and 2, and/or VZV without detectable reactivity against this new agent. Likewise, sera were found which reacted against this new virus agent without reactivity against any of the known human herpesviruses. A series of studies indicate that this new cytopathogenic agent replicates in T-cells. This virus appears to be different than the other human herpesviruses and has thus been designated human herpes virus - type 6 (HHV-6).

R37.31

GENITAL HERPES INFECTION IN THE POPULATION OF MADRID

P. Pérez Breña[1]., C. Echevarría[1]., S. Carlos[1]., B.Melendez[2]

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The frecuency of HSV infection among sexually transmited disease (STD) in the Madrid population is evaluated. Genital samples were obtained from two family planning centers and two STD clinics, during the last three years. Samples were cultured in human diploid fibroblast cells. Identification and typing was performed by IFA with monoclonals antibodies.

The percentage of positivity in the whole samples was 1,8%, ranging from 18% in the group suspected of HSV genital infections to 0,5% among the asymptomatic controls. Dividing the analised population by their sexual habits, the percent of positives is 5 to 9 fold higher in the more promiscuous group. High risk groups (homosexuals,prostitutes, prison population) are analised and compared with other types of population. Distribution by ages, types, incidence of neonatal herpes and other markers are considered, concluding that the prevalence of HSV genital infection in Madrid is low.

R38.1

GLYCOSYLATION ANALYSIS OF GLYCOPROTEIN 120 (GP120) FROM HUMAN IMMUNODEFICIENCY VIRUS (HIV).

Ch. Holschbach, G. Hunsmann, J. Schneider, and H. Geyer. Institute of Biochemistry, University of Giessen, Giessen and German Primate Center, Göttingen, FRG.

HT-H9 cells, persistently infected with HIV, were metabolically labeled with [2-^{3}H] mannose or [2-^{3}H] glucosamine. Gp120 was isolated from the supernatant after centrifugation of the cells or from cell lysates by immunoprecipitation and further purified by preparative SDS-gel electrophoresis. Following digestion of the glycoprotein with protease from S.aureus (V8), oligosaccharide side chains were sequentially liberated by treatment with endoglycosidase H and peptide:N-glycanase F. Oligosaccharides released were separated from residual (glyco)peptides by gel filtration and reversed-phase HPLC, were fractionated according to fucose content and charge, and were subfractionated according to size by different HPLC methods. The individual oligosaccharide species obtained were characterized by comparison of their chromatographic behavior with standard oligosaccharides, by digestion with exoglycosidases and by acetolysis. Our results demonstrate significant differences in the glycosylation of intracellular and secreted gp120. Structures of the major carbohydrate substituents will be proposed.

R38.2

CHARACTERIZATION OF AN ISOLATE OF HUMAN IMMUNODEFICIENCY VIRUS IN ASIA. W. T. LIU

National Yang-Ming Medical College and Laboratory of Clinical Virology, Veterans General Hospital, Taipei, Taiwan

The first isolate of human immunodeficiency virus was identified in Taiwan (probably in Asia) in February, 1986 from an autopsy-proved case of acquired immunodeficiency syndrome in Chinese.

This virus isolate was characterized in general by syncytial cytopathic effect of co-cultured CEM cells and specific immunofluorescent viral antigens on the co-cultured cells. The typical type D virus particles with a cylindrical core were seen in thin-sections of the lymphocytes by electron microscopy. Reverse transcriptase activity was also detected in the supernatant of the culture 14-21 days after co-culture in primary isolation.

Further propagation of the virus was done with CEM and H9 cell lines and used for advanced studies. Therefore, the density of the virion, viral RNA and viral DNA after reverse transcription were analysed. Western blotting was also applied for evaluation of the viral proteins during replication in the lymphoid cell lines.

R38.3

SELECT LECTINS INACTIVATE HIV IN VITRO. W. Edward Robinson, David C. Montefiori and William M. Mitchell, Vanderbilt University, Nashville, Tennessee, USA.

To evaluate the potential role of oligosaccharides in the attachment of HIV to the T4 receptor, we examined the effect of several lectins on viral infectivity. Lectins tested were BS-II, lentil, wheat germ agglutinin (WGA), hairy vetch, red marine algae, phytohemagglutinin-P (PHA), concanavalin-A (Con-A), and succinyl concanavalin-A (sCon-A). We found that at 500 nM concentrations Con-A and sCon-A preincubated with virus completely inhibited virus expression in target C3 cells as determined by indirect immunofluorescence (IIF) and reverse transcriptase assay (RT). Lentil and PHA greatly diminished viral infectivity (IIF and RT <20% of control) while WGA only slightly inhibited the virus (RT and IIF <60% of control). BS-II, hairy vetch, and red marine algae failed to inactivate the virus at all (RT and IIF >80% of control). We further found that monovalent sCon-A can prevent HIV infection of target cells significantly at a concentration of 400 nM. Since there is no relationship between lectin size and degree of inhibition of HIV infectibility, the observed antiviral activity appears to be related to lectin oligosaccharide specificity rather than simple, non-specific stearic hindrance. Thus, these findings indicate that the oligosaccharide side chains of the HIV envelope proteins may be involved in the attachment of the virus to the T4 receptor of T-helper lymphocytes and that modulation of HIV oligosaccharides may be a useful target for the development of chemical anti-HIV agents.

R38.4

MODIFICATION OF HIV N-GLYCOSYLATION BY THE α-MANNOSIDASE INHIBITOR SWAINSONINE. David C. Montefiori, W. Edward Robinson and William M. Mitchell, Vanderbilt University, School of Medicine, Nashville, Tennessee, USA.

The two envelope glycoproteins (gp41 and gp120) of human immunodeficiency virus (HIV) serve functions obligatory to the pathogenesis of HIV including attachment of virus to target cells and syncytium formation. Swainsonine is an indolizidine alkaloid found in certain legumes and molds and alters protein N-glycosylation through its potent reversible inhibition of α-mannosidase II (Broquist, H., Ann. Rev. Nutr. 5, 391-409, 1985). Our interest has been to investigate the significance of N-glycosylation in HIV pathogenesis using swainsonine and other inhibitors of glycoprotein processing as tools. We have found that swainsonine affects N-glycosylation of HIV glycoproteins without affecting virus yields. The concentrations of swainsonine utilized in these studies (1-10 µg/ml) had no effect on cell division or RNA and protein synthesis. Synthesis of virus in the presence of swainsonine was confirmed by reverse transcriptase activity in culture fluids and by density gradient centrifugation of [^{35}S] methionine labelled virus. Results of [^{3}H] mannose labelling experiments and SDS-PAGE/fluorography demonstrated that swainsonine causes a reduction in the molecular weight of the HIV envelope gp120 reducing it to a gp110 molecule.

R38.5

A Murine Monoclonal Antibody (MAb) to a Synthetic Peptide Is Reactive with Proteins Coded for by the HTLV-III(HIV) 3'-orf Gene. P. Durda¶ A. Jenoski¶ H. Rabin*, A. Fisher**, F. Wong-Staal**, and R. Gallo**. *E.I. du Pont de Nemours & Co., N. Billerica, MA, USA, **NCI/NIH, Bethesda, MD, USA

MAbs to putative gene products can be developed using short amino acid (aa) sequences predicted to be present in such proteins. A MAb, designated 2912, was developed to a synthetic peptide representing amino acids 15-27 of 3'-orf (AVRERMRRAEPAA). In Western blots (WB) using reduced lysates of purified HTLV-3B virus MAb 2912 reacted with proteins having Mr = 27 and 14 kD. MAb 2912 showed no reactivity in WB with proteins present in comparable lysates from noninfected cells. In WB analyses of proteins produced in H9 or COS cells following introduction of the molecular clones pHXB3, pHXB2D, or six different 3'-orf deletion mutants derived from pHXB2D(A.Fisher et al. Science 233:655, 1986), only pHXB3 yielded a specific signal (at 28.5 kD). pHXB3 is predicted to code for a full length 3'-orf product having alanine at aa position 15, whereas pHXB2D codes for a shortened 3'-orf product of 133 aa's having a threonine at position 15. In summary, a MAb developed to a short synthetic peptide recognizes 2 putative gene protein products in viral lysates, one full length and another of 14kD presumably due to a stop codon at nucleotide positions 8743-8745 which is present in some HTLV-III clones. It also can apparently discriminate between the different gene products coded for by the molecular clones pHXB3 and pHXB2D.

R38.6

HIV-SPECIFIC CYTOTOXIC T CELLS AS MODELS FOR INVESTIGATIONS ON ACTIVITIES RELATED TO HUMAN IMMUNODEFIENCY VIRUSES (HIV). E. N. Arida

National Research Center, Dokki, Cairo, Egypt.

HIV-specific cytotoxic T cells were developed by multistep treatments of human T cells with factors influencing gene derepression & oxidized DNA fragments (ODF) prepared from HTLV-3 positive blood specimens. These cells possessed specific susceptibility to HIV-related materials indicating genome integration of an HIV provirus. The influence of different biological and chemical materials were studied employing these cells. This included T cells or ODF obtained from HTLV-3 positive blood (I), murine sarcoma viral genes(II) formalin-inactivated viral concentrates (III), live attenuated viral vaccines (IV), Lectins (V), immune complexes (VI), polynucleotides (PN) (VII), PdN including hybrids (VIII), Azathymidine (AZT) (IX), AZU, AZG, AZC (X), singly or combined.

I had specific cytotoxic effect. II & IX had resulted in loss of the specific cytotoxic responses with restricted persistence with IX. Lectins had no influence and the cells proliferated in their absence indicating HIV associated changes of T cell response. These findings point to the value of these cells in studies related to HIV.

R38.7

EVOLUTION AND SEQUENCE DIVERGENCE OF HUMAN IMMUNODEFICIENCY VIRUS TYPE I (HIV-1) AS DETERMINED BY COMPUTERIZED STATISTICAL ANALYSIS OF RESTRICTION ENZYME POLYMORPHISMS.

J. Campione-Piccardo, Health and Welfare Canada, L.C.D.C., Molecular Virology Laboratory, Ottawa, Ontario, Canada K1A 0L2.

The available evidence on sequence divergence in strains of human immunodefficiency virus type 1 (HIV-1) has been obtained mainly from visual inspection of restriction enzyme maps, or from analysis of a limited number of sequenced viral genomes. The computerized statistical analysis being reported here, allowed the simulatenous comparison of all genomes with known maps or sequence data. New specialized algorithms were specifically developed and implemented for this statistical analysis. Iterative procedures were used to establish from published maps the presence of similar or new sites in all the strains included in the study. Estimators for proportion of mismatches and genomic heterogeneity were used to estimate the sequence variability within and among strains of different procedence, as well as the sequence variability within different regions of the viral genome. In addition, different clustering methods were explored to estimate the phylogeny of the virus. These studies revealed that statistical analysis of restriction enzyme cleavage data can provide a powerful alternative for the analysis of sequence divergence among large samples of related viral genomes.

OPEN POSTERS

AFFICHES OUVERTES

OP1.1

WILD DUCKS IN ALBERTA HARBOUR ALL OF THE KNOWN INFLUENZA A VIRUSES. R.G. Webster[1], V.S. Hinshaw[2], Y. Kawaoka[1], A. Nestorowicz[1], W.J.L. Bladen[3] and B. Turner[4]. [1]St. Jude Children's Research Hospital, Memphis, TN, [2]University of Wisconsin-Madison, [3]Horsehead Sanctuary, Grasonville, Maryland, [4]Canadian Wildlife Service, Edmonton

Surveillance of wild ducks in Alberta over 11 years has established that these birds are the natural reservoir of all known influenza A virus subtypes. Each August up to 30% of young birds shed high concentrations of virus in their feces, but show no disease signs. Viruses are spread by the fecal-oral route.

Wild ducks also have the potential to be reservoirs of H5 and H7 influenza viruses some of which can be highly pathogenic in chickens and turkeys yet cause no disease signs in ducks.

Sea birds also harbour a wide range of influenza A viruses and the extent of this reservoir and interspecies transmission of these viruses to avian and mammalian species is not yet resolved.

OP1.2

DEFECTIVE INTERFERING PARTICLES ASSOCIATED WITH AN INFLUENZA VIRUS ISOLATED FROM A NATURAL INFECTION. T. Chambers, and R. Webster. Department of Virology and Molecular Biology, St. Jude Children's Research Hospital, Memphis, TN, USA.

The A/Chicken/Pennsylvania/1/83 influenza virus, isolated from a respiratory infection of chickens, is an avirulent H5N2 virus containing subgenomic RNAs (Bean et al., J. Virol. 54:151-160, 1985). We show that defective interfering particles are present in this virus population. It had a low PFU/HAU ratio, and produced interference with standard virus multiplication in infectious center reduction assays. Subgenomic RNAs were identified as internally deleted influenza polymerase gene RNAs. This virus protected chickens from lethal H5N2 influenza. Protection appeared to be due to direct inhibition of virulent virus multiplication. Additionally, subgenomic RNAs derived from polymerase RNAs were detected in 5 of 18 RNA preparations from animal influenza virus isolates. Therefore, defective interfering particles are sometimes produced in natural influenza infections, not just under laboratory conditions. These particles may be capable of suppressing the pathogenic effect of virulent virus infections in nature.

OP1.3

MUTANTS OF INFLUENZA VIRUS DEFECTIVE IN THE DIRECTED TRANSPORT OF THE HEMAGGLUTININ

W.Garten[1], C. Will[1], W. Schuy[2], K. Kuroda[1], K. Buckard[2], C. Scholtissek[2], and H.-D. Klenk[1]

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[2]Institut für Virologie, Justus-Liebig Universität, 6300 Gießen, FRG.

Two groups of mutants of FPV with ts defects in hemagglutinin transport have been obtained. With the first group, transport is arrested in the rough endoplasmic reticulum, with the other group it is blocked between the Golgi apparatus and the plasma membrane. Sequence analyses carried out on these mutants and their revertants revealed that the transport defects are due to single point mutations. The available evidence indicates that, with the early transport mutants, the resulting amino acid exchanges interfere with trimerization. The alterations responsible for the late transport defects involve exchanges of charged residues located at the surface of the hemagglutinin stalk which appear not to interfere with trimerization.

OP1.4

CARBOHYDRATES OF INFLUENZA VIRUS. STRUCTURE OF THE OLIGOSACCHARIDES LINKED TO ASPARAGINES 406 AND 478 IN THE HEMAGGLUTININ OF FOWL PLAGUE VIRUS, STRAIN DUTCH. R. Geyer[1], S. Diabate[1], H. Geyer[1], H.-D. Klenk[2], H. Niemann[2], and S. Stirm[1]. [1]Biochemisches Institut, Friedrichstrasse 24, D-6300 Giessen, FRG; [2]Institut für Medizinische Virologie, Frankfurter Strasse 107, D-6300 Giessen, FRG

Fowl plague virus, strain Dutch, was metabolically labelled with D-[2-^{3}H]mannose, or with D-[6-^{3}H]glucosamine, and the small subunit (HA_2; 0.8 mg in total) of the viral hemagglutinin was isolated by preparative sodium dodecyl sulfate-polyacrylamide-gel electrophoresis. After proteolytic digestion, the radioactive oligosaccharides were sequentially liberated from the glycopeptides by treatment with different endo-β-N-acetylglucosaminidases and with peptide:N-glycosidase or, finally, by hydrazinolysis. In this manner, four groups of glycans could be obtained by consecutive gel filtrations and were subfractionated by HPLC. The structures of the individual oligosaccharides were analyzed by micromethylation, by acetolysis or by digestion with exoglycosidases. The major species amongst the glycans at Asn-406 of HA_2 were found to be Manα1-2Manα1-3 (Manα1-2Manα1-6) Manα1-6 (Manα1-2Manα1-2Manα1-3) Manβ1-4GlcNAcβ1-4GlcNAc and Manα1-3(Manα1-2Manα1-6)Manα1-6(Manα1-2Manα1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc, while the major substituents at Asn-478 were found to be GlcNAcβ1-2Manα1-3(GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAc (lacking, in part, one of the outer GlcNAc-residues) and GlcNAcβ1-2Manα1-3(Galβ1-4GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAc. Thus, the carbohydrate substituents of the two N-glycosylation sites of HA_2 from fowl plague virus, strain Dutch, reflect different stages of oligosaccharide processing during glycoprotein biosynthesis.

OP1.5

CODING AND INFECTION STRATEGY OF THOGOTO VIRUS.

D. Staunton, C.R. Davies, and P.A. Nuttall. NERC Institute of Virology, Oxford, OX1 3SR, United Kingdom.

Thogoto is a tick-borne virus originally isolated from a mixed population of Boophilus and Rhipicephalus ticks from cattle in Kenya. It has since been found throughout central Africa and southern Europe. Thogoto virus is a candidate member of the Orthomyxoviridae; it buds from the plasma membrane and has a segmented single strand RNA genome.

A Sicilian isolate of Thogoto was purified for electron microscopy and RNA extraction. RNA segment profiles of the Sicilian and prototype isolates showed no discernible difference in segment mobilities.

cDNA recombinants, produced from RNA of the Sicilian isolate, were sequenced. The protein sequence was deduced and compared with available protein sequences of Influenza A, B and C.

Laboratory studies were undertaken to examine the interactions between Thogoto and a tick vector R.appendiculatus. The virus pathway in infected ticks was studied using two techniques. Infected tick sections were labelled using immunofluorescence to determine the timecourse of viral antigen presence in different tick organs; and a cDNA recombinant was biotin labelled to probe tick sections for the presence of viral RNA.

OP1.6

EFFECT OF HOST IMMUNITY ON THE TRANSMISSION OF THOGOTO VIRUS, A TICK-BORNE INFLUENZA LIKE VIRUS. Jones, L.D. and Nuttall, P.A. NERC Institute of Virology, Mansfield Road, Oxford OX1 3SR, United Kingdom.

Thogoto (THO) virus, unlike other members of the family Orthomyxoviridae, is an arbovirus and is biologically transmitted by the Brown Ear Tick Rhipicephalus appendiculatus. Thogoto virus is widespread throughout central Africa and has been isolated from the Middle East and Southern Europe, with evidence that it is of veterinary and medical significance.

Two forms of host immunity may affect tick-borne virus transmission: (i) immunity to the virus, and (ii) resistance to tick infestation. The first was examined by interrupted feeding (ticks partially engorged on a viraemic host allowed to complete their blood meal on a virus-immune host, and vice versa), and transtadial persistence (feeding on a viraemic host, moulting to the next developmental stage, then feeding on a virus-immune host, and vice versa). The second was investigated by feeding infected/uninfected ticks on infected/uninfected hosts resistant to tick infestation, and then determining the effect on virus acquisition and transmission by the ticks. Results of these experiments have significance for the epidemiology of arboviruses.

OP1.7

COMPLETE NUCLEOTIDE SEQUENCE OF AN INFLUENZA HEMAGGLUTININ OF SUBTYPE H10.

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The nucleotide sequence of the hemagglutinin of influenza virus A/chick/Germany 49 (H10N7) (virus N) was determined. The hemagglutinin has a total length of 561 amino acids and contains 6 potential carbohydrate attachment sites, 5 of which are glycosylated. The cleavage site of the hemagglutinin of virus N which is the prototype of an apathogenic avian strain shows a single arginine connecting HA_1 and HA_2, like other hemagglutinins with restricted cleavability. Comparison of the H10 hemagglutinin with subtypes H7, H3, H1, H2, and H5 revealed 63%, 51%, 44%, 43%, and 42% sequence homology, respectively.

OP1.8

STRUCTURAL STUDIES OF INFLUENZA B HEMAGGLUTININ AND NEURAMINIDASE. P. J. Bossart, G. M. Air. Department of Microbiology, University of Alabama at Birmingham, Birmingham, AL USA.

We are determining the molecular basis of antigenic variation in influenza B viruses as compared with antigenic drift in influenza A. Considerable antigenic diversity exists in the two surface antigens, which in influenza A and B are the hemaglutinin (HA) and neuraminidase (NA). In order to better understand the mechanism(s) of antigenic diversity, structural information concerning the two surface antigens is essential. Both the HA (B/HK) and NA (B/Lee) obtained from a high growth reassortment virus were candidates for crystallization. Crystallization has been highly successful for B/Lee NA. Crystals have been reproducibly grown to a size suitable for study by X-ray diffraction. The crystals are stable in the X-ray beam and diffract to greater than 4 Å resolution. Space group determination in addition to unit cell dimensions are presented. Because of the difficulty associated with growing suitable crystals of the HA, oligonucleotide directed, site-specific mutagenesis has been employed as a tool to probe the structure of the HA molecule. Mutant proteins are expressed [illegible]V40 late replacement vector and changes in struc[illegible]ored with monoclonal antibodies.

OP1.9

MECHANISM OF INFLUENZA VIRUS NEUTRALIZATION ON CILIATED CELLS OF THE TRACHEA. M. Outlaw, N. J. Dimmock.
Department of Biological Sciences, University of Warwick, Coventry, CV4 7AL, England.

Mechanisms of influenza virus neutralization have, until now, been studied using tissue culture cell systems. Such cells are dedifferentiated and often unlike the cells of the natural target tissue. The dependence of neutralization on the host cell has recently been demonstrated with picorna and bunya viruses, and stresses the need to take the type of cell into account in such studies.

Here we describe an organ culture system derived from mouse trachea, which has been used to study the mechanism of neutralization by mouse IgG antibodies. We find that attachment of radiolabelled influenza virus to ciliated epithelial cells is affected to a varying degree depending on the proportion of virus and antibody in the neutralization mixture.

OP1.10

MONOCLONAL ANTIBODIES DISCRIMINATE SUPERFICIAL FROM INTERNAL M-PROTEIN EPITOPES OF INFLUENZA VIRION. L. Joassin, C. Vincenzotto, J.M.Cloes, M. Bouchet, M.Reginster, University of Liège, Laboratory for Medical Virology, B23, 4000 LIEGE, BELGIUM.

Four anti type A M-protein monoclonal antibodies have been used to investigate the expression of influenza A epitopes on the surface of the virions. We developped a specific and sensitive competition ELISA where intact virions, dodecylsulfate disrupted virions and spikeless particles obtained after proteolytic treatment with caseinase C were used to test their ability to inhibit the reaction between the monoclonal antibodies and pure M-protein. Intact or SDS disrupted virions prevented three monoclonal antibodies from reacting with the M-protein. Spikeless particles also inhibited the specific binding of two antibodies, whereas the other fourth antibody was inhibited by contact with SDS disrupted particles only.

Data presented show that at least three distinct M-protein epitopes were detected, of which at least two, are exposed on the surface of intact virions. Of these two epitopes, one is inactivated by the proteolytic treatment. The third epitope could only react with its monoclonal antibody when the virus particles were solubilized with SDS.

OP1.11

RADIATION INACTIVATION ANALYSIS OF INFLUENZA AND SENDAI MEDIATED FUSION AND HEMOLYSIS, J. Lenard, S. Gibson and K. Bundo-Morita, UMDNJ-Robert Wood Johnson (formerly Rutgers) Medical School, Piscataway, NJ 08854-5634.

The target sizes for inactivation of the fusion and hemolysis activities of influenza and Sendai virus glycoproteins were determined by radiation analysis. Fusion with erythrocyte ghosts was determined after loading virions with octadecylrhodamine B chloride (R18). Fusion with cardiolipin (CL) liposomes was determined by measuring relief of resonance energy transfer between fluorescent donor and acceptor phospholipids present in the liposomes. Influenza had a similar target size for fusion with ghosts and CL liposomes, ca. 75 kDa, corresponding to a single HA monomer. Sendai had a similar target size of ca. 73 kDa for fusion with ghosts, but had a much larger, more complex target size for fusion with CL liposomes: the inactivation curve had a pronounced shoulder, suggesting a functional unit composed of several separate entities. Sendai, therefore, fuses with CL liposomes and ghosts by different mechanisms, while influenza may use the same mechanism for both. Hemolysis target sizes differed from fusion target sizes for both viruses, suggesting that leakage may be actively mediated by an ensemble of viral proteins different from those responsible for fusion.

OP1.12

GENOME EVOLUTION OF HUMAN EPIDEMIC INFLUENZA VIRUSES

N. J. Cox and A. P. Kendal. WHO Collaborating Center for Influenza, Center for Infectious Diseases, Centers for Disease Control, Atlanta, GA 30333.

T_1 oligonucleotide mapping has been performed on total viral RNA of representative epidemic influenza A virus isolates obtained during two H1N1 epidemic periods (1946-1957 and 1977-1986) and during the H3N2 epidemic period (1975-1986). During each epidemic period there were intervals during which a gradual accumulation of oligonucleotide changes (4-6/year) were observed as well as intervals during which an apparent "burst" of changes (20-30/year) occurred in the virus genome. Only once was this "burst" associated with the emergence of inter-subtype reassortant. Evolutionary relationships were deduced for epidemic viruses during each epidemic period using both T1 oligonucleotide mapping and DNA sequencing data. Factors that may affect the rate of evolution of influenza virus genomes are discussed.

OP1.13

TEN-YEAR STUDY OF INFLUENZA SUBUNIT VACCINE IN PATIENTS WITH NEUROMUSCULAR DISEASES. T. Yokoi[1], S. Miyoshino[2], M. Kaji[3]. Department of Bioclimatology and Medicine, Medical Inst. of Bioregulation, Kyushu University[1], Department of Pediatrics, Nishi-Beppu National Hospital[2], Beppu, First Department of Internal Medicine, Kurume University[3], Kurume, Japan.

In total 1,473 patients with neuromuscular diseases, including 1,016 patients with Duchenne muscular dystrophy, and 3,768 healthy controls received bivalent(H3 and B or H1 and B) or trivalent(H1, H3 and B) influenza subunit vaccine subcutaneously in the 10-year period from 1976 to 1985. Before, 1 month after, and also 3 months after the vaccination, serum haemagglutination-inhibiting (HI) antibody titers were measured and the incidence of reactions to the vaccine was recorded for 3 days after inoculation.

In the patient group, 22%-65% of vaccinees showed a four-fold or greater rise in HI antibody titer to H3, 30%-70% to H1 and 11%-41% to B. On the basis of antibody measurement, the patient group showed a similar qualitative and quantitative response to that of the controls. The reactions to the vaccine were mild and were observed in less than 3% in both groups.

We conclude that it is safe to vaccinate patients with neuromuscular diseases with an influenza subunit vaccine and a good antibody response can be induced.

OP1.14

FUNCTIONAL STUDIES OF THE CAP-BINDING PROTEIN, pb2 OF INFLUENZA VIRUS USING DIRECTED ANTISERA. V.C. Blok, S.C. Inglis. Division of Virology, Department of Pathology, University of Cambridge, Cambridge, U.K.

A panel of rabbit antisera directed against five different regions of the cap binding protein of influenza virus, pb2, have been raised. Restriction enzyme fragments representing the entire coding sequence of pb2 were cloned into the pEX vectors developed by Stanley and Luzio, 1984, and expressed in E. coli as carboxy-terminal fusions of ß-galactosidase. These fusion proteins were purified and used as immunogens for rabbits. Antisera were obtained which reacted with pb2 protein from purified virus in Western blots and in immune precipitations with pb2 protein prepared in the cell-free translation system, rabbit reticulocyte lysate. The pb2 mRNA translated *in vitro* was derived from a cDNA copy of pb2 vRNA cloned into pT7.2 vector downstream of the bacteriophage T7 RNA polymerase promoter. The antisera have been used for functional studies of the pb2 protein *in vitro*.

OP1.15

HOST CELL-MEDIATED VARIATION IN H3N2 INFLUENZA VIRUSES. J.M. Katz, C.W. Naeve and R.G. Webster. Department of Virology and Molecular Biology, St. Jude Children's Research Hospital, Memphis, TN, USA.

Human type A (H3N2) influenza viruses grown in embryonated eggs frequently possess antigenically distinguishable hemagglutinin (HA) compared to virus from the same source grown in mammalian Madin Darby Canine Kidney (MDCK) cells. These findings have raised concern regarding the suitability of eggs for cultivation of influenza viruses used in epidemiological studies and vaccine production.

We have investigated the molecular basis of host-mediated antigenic variation and its importance in the induction of immunity by the variants. When cultured in eggs, a subpopulation of virus is selected which typically possesses a single amino acid change in the HA compared to virus from the same source grown in MDCK cells. This change is located at the tip of the HA molecule, proximal to the receptor-binding site.

Protection studies indicate that the antigenic and molecular changes in the HA which accompany host cell selection are irrelevant in terms of immunity when live egg- or MDCK-grown vaccines are used to protect ferrets from infection with virus grown in either host cell type. However, differences in efficacy between mammalian cell-grown and egg-grown vaccines are observed when formalin-inactivated viruses are used to vaccinate ferrets.

OP1.16

ANTIBODY-MEDIATED REPLICATION OF INFLUENZA NWS VIRUS IN A MACROPHAGE-LIKE CELL LINE, P388D1. H. Ochiai, M. Kurokawa, K. Hayashi, S. Niwayama. Department of Virology, Toyama Medical and Pharmaceutical University, Toyama, Japan.

The entry pathway of influenza NWS virus into P388D1 cells was examined by flow cytometric analysis using FITC-labeled virus. The attachment of normal rabbit serum-treated virus(NS-V) to neuraminidase(NA)-treated cells was noticeably limited, compared with that to the untreated cells. However, rabbit antiserum-treated virus(AS-V) could equally attach to both cells with higher efficiency. The virus coated with Fab fragment prepared from the anti-virus IgG, which retained HI activity to NWS virus, could not attach. These data suggest that NWS virus can infect P388D1 cells in one of two ways : via NA-sensitive viral receptors or via Fc receptors. In addition, the virus replication after internalization via one of two ways was examined by plaque assay on MDCK cells. The NS-V could replicated in the untreated cells, but not in the NA-treated cells(<10 PFU/ml at 24h p.i.). On the other hand, the replication of AS-V in the NA-treated cells was dependent on the concentrations of antibody, *i.e.*, this was markedly enhanced at subneutralizing concentrations of antibody($4x10^3$ PFU/ml at 24h p.i.), but at the higher concentrations or in the absence of antibody, little or no replication was observed. These data suggest that antibody-mediated virus replication could be regulated by some factor in the polyclonal antibody depending on the concentrations of antibody.

OP1.17

ANTIGENIC ANALYSES OF RECENT INFLUENZA A(H1N1) VIRUSES. J.S. Robertson, P.J. Yates, J.S. Bootman National Institute for Biological Standards and Control, South Mimms, Herts, UK EN6 3QG

Antigenic and HA structural differences have been described previously for influenza viruses derived from the same clinical specimen but passaged either exclusively in MDCK cells or adapted to growth in hens' eggs. We have constructed an operational antigenic map of a 1983 A(H1N1) virus isolated and propagated exclusively in MDCK cells. Two distinct antigenic sites could be defined corresponding to the previously defined sites Sa and Sb. Variants resistant to a third group of monoclonal antibodies could not be derived on MDCK cells until an additional substitution occurred in the HA molecule adjacent to the receptor binding site. This could be achieved by passage of the virus in eggs prior to selection of antigenic variants in eggs or in MDCK cells. These and other analyses indicate the importance of HA residue 225 in defining receptor binding site specificity in A(H1N1) viruses.

OP1.18

INTRANASAL INOCULATION OF ETHER-TREATED TRIVALENT INFLUENZA VACCINE M.Nakayama, T.Kitano, A.Oya(NIH), K.Aoyama(Metropolitan Medical Center of the Severely Handicapped), Y.Takeuchi(Chiba Prefecture Serum Institute), M.Kimura(Tokai University), H.Mizutani (Kanto Teisin Hosp.), Y.Yamazi(Nippon Medical School), Tokyo. Japan.

Antibody responces after intranasal inoculation (i.n.) and subcutaneous (s.c.) inoculation of influenza vaccine were compared. Vaccine used was ether-treated trivalent vaccine and contained A/Philippine/2/82(H3N2),A/Bangkok/10/83(HINI) and B/Singapore/222/79.

Fifteen volunteers were inoculated i.n. three times with 0.3ml vaccine with 4 weeks interval. S.c.inoculation was performed against 66 volunteers twice with 0.5ml vaccine with 4 weeks interval. Four to seven weeks after the final vaccination, sera were collected and the HI antibody titer was examined. In i.n. group, IgA ELISA titer in the nasal secretions was also examined.

In s.c. group, HI titer increased by 2.2 (B) to 4.5times (H3N2), whereas in i.n. group, HI titer increase was much higher and by 2.5 (B) to 10.5 times(HINI). From this result it was concluded that i.n. immunization was superior to s.c. to produce HI antibody in sera. Determination of IgA ELISA titer in the nasal secretion will be also reported.

OP1.19

IN VITRO STUDIES OF RECOMBINANT MX PROTEIN. J.H. Condra[1], M.E. Davies[1], M.J. Berry[1], I. Julkunen[2], R.M. Krug[2], and R.J. Colonno[1], [1]Department of Virus & Cell Biology, Merck Sharp & Dohme Research Laboratories, West Point, PA 19486, U.S.A. and [2]Graduate Program in Molecular Biology, Memorial Sloan-Kettering Cancer Center, New York, NY 10021, U.S.A.

The mouse Mx^+ gene encodes an interferon-induced 72kDa nuclear protein that exerts a potent influenza-specific antiviral effect. [Review: O. Haller, Curr. Topics Micro. Immunol. 92:25 (1981)]. When Mx-expressing cells are infected with influenza, viral primary transcription is inhibited, suggesting that Mx protein blocks viral mRNA synthesis [R.M. Krug et al., J. Virol. 56:201 (1985)].

In order to define the mechanism of Mx action, we have cloned a full-length cDNA copy of the Mx gene and expressed the protein in a variety of systems. By translating in vitro Mx transcripts in rabbit reticulocyte lysates, radiochemically-pure [^{35}S]-Mx protein is synthesized that immunoprecipitates with anti-Mx antiserum.

As a source of purified Mx protein for biochemical studies, we have expressed Mx as a fusion protein in E. coli and have raised antibodies against the purified protein. We have also achieved high-level expression of Mx as a nonfusion protein in S. frugiperda cells, using baculovirus vectors. The effect of recombinant Mx protein on influenza virus transcription is being investigated.

OP1.21

LABORATORY INVESTIGATIONS ON INFLUENZA VIRUSES DIFFUSION IN ITALY IN THE LAST THREE SEASONS.

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Department of Virology,Istituto Superiore Sanità,Rome,Italy

Laboratory investigations on influenza viruses diffusion in Italy during the seasons 1985-86-87 are presented. A/Philippines/2/82 like strain predominated in the winter 1985(66%); a new variant,showing some antigenic differences to Philippines,has been isolated(26%) in Rome and in several towns of Italy. This variant(A/Roma/6/86) has been isolated also in 1986 (20%),when strains similar to A/Christchurch/4/85 predominated. The results of antibody surveys to currently circulating viruses,performed on a large sample of people,in the summer 1986(when influenza virus ceased to be detected) demostrated the mild diffusion of the new antigenic variant A/Taiwan/I/86-HINI,not isolated during the winter.
The incidence of influenza like illness in the years 1986 - 1987 slightly increased since mid January ;the A/Taiwan/I/86 like strain is the only influenza virus isolated until February.

OP1.20

ENZYMATIC MECHANISMS OF TRANSCRIPTION AND REPLICATION OF INFLUENZA VIRAL RNA. K.Nagata, K.Takeuchi, A.Honda, K.Ueda, A.Kato, and A.Ishihama. Department of Molecular Genetics, National Institute of Genetics, Mishima, Shizuoka, Japan.

We have taken two approaches to study mechanisms of transcription and replication of influenza viral RNA. First, the RNP complex, which contains three P proteins(PB1,PB2 and PA), NP protein and virion RNAs, and the P-RNA complex devoid of the NP protein have been isolated from detergent-solubilized virions. The P-RNA complex supports the endonucleolytic cleavage of capped RNA, initiation of RNA synthesis and at least partial elongation reaction, whereas the efficient production of full-sized RNAs takes place only in the RNP complex, suggesting that the NP protein is required for efficient elongation. To confirm this,transfection experiments of the RNP and the P-RNA complexes are being carried out.

Second, we have developed an in vitro transcription and replication system using nuclei isolated from virus-infected cells. In this system, two species of positive-sense RNA transcripts, i.e. mRNA and cRNA(complete copy of vRNA), are synthesized. In the presence of high concentrations of salt, the synthesis of mRNA predominates. Nuclear salt extracts selectively synthesize cRNA, whereas salt-treated nuclei synthesize mRNA. Addition of the former to the latter leads predominant synthesis of cRNA, suggesting that a switching factor(s) involved in synthesis of mRNA to cRNA is present in the nuclear extracts. To identify the regulatory factor(s), fractionation and reconstitution experiments are in progress.

OP1.22

INFLUENZA VIRAL POLYMERASE PROTEINS: SUBCELLULAR LOCATION, ASSEMBLY AND INTERACTION WITH VIRAL RNA. R. K. Akkina[1], T.M. Chambers[1], D.R. Londo[2], A.T. Mimiaga[1], and D.P. Nayak[2], Department of Microbiology, Colorado State University, Fort Collins, Colorado, 80523[1], and Department of Microbiology and Immunology, UCLA School of Medicine, Los Angeles, California, 90024[2], USA.

Influenza viral RNA is transcribed and replicated by a virus specified polymerase complex composed of three polypeptides namely PB_1, PB_2, and PA. We have expressed parts of the cDNAs of polymerase genes in bacteria as fusion proteins and made monospecific antisera to each polymerase protein. Intracellular location, nuclear transport and complex formation among the three polymerases in infected cells are studied with the aid of these monospecific antisera. Differential accumulation of these polymerases in nucleus vs. cytoplasm has been found in early stages of infection. Accumulation of PA in nucleus is delayed compared to PB_1 and PB_2. PB_1 protein when expressed alone by the use of SV40 late promoter expression vector, accumulated in the cell nucleus suggesting that it contains intrinsic signals for nuclear transport. Our results also indicate that RNP-polymerase complexes that get incorporated into mature virus particles are assembled in the nucleus.

OP2.1

LTR STRUCTURAL ALTERATIONS OF ACQUIRED MMTV PROVIRUSES FROM T-CELL LYMPHOMAS. C-L. L. Hsu, C. Fabritius, and J. Dudley. Department of Microbiology, University of Texas at Austin, Austin, TX, USA.

Similar to mouse mammary tumor virus (MMTV)-induced mammary carcinomas, many murine T-cell lymphomas acquire additional integrated copies of MMTV. Southern blot analysis indicates that the acquired MMTV copies in C57BL/6 (B6) lymphomas are very polymorphic and originate from endogenous MMTV DNA. Experiments with cloned MMTV proviral DNA from these tumors indicate that all of the acquired copies contain a deletion within the U_3 region of the LTR which encompasses the 5' end of the glucocorticoid regulatory element (GRE) and the 3' end of the LTR long open reading frame. In vivo the lymphomas express high levels of MMTV RNA from the truncated proviruses but not from the three full-length endogenous proviruses. The structure and transcriptional activity of these variant LTRs will be presented.

OP2.2

VARIANTS OF FRIEND ERYTHROLEUKEMIA VIRUS IN GENETICALLY RESISTANT MICE: A HISTOLOGICAL STUDY. M.L. Stevens, R.F. Laherty, R.W. Geib. I.U. Dept. of Pathology, I.U. School of Medicine, Terre Haute Center for Medical Education, Terre Haute, IN, U.S.A.

Friend virus (FV) is an acutely oncogenic retrovirus which induces rapid eryhtroleukemia in susceptible mice ($FV\text{-}2^{ss}$). FV is a complex involving a replication competent helper virus (F-MuLV) and a replication defective spleen focus forming virus (SFFV). Upon passage of this virus through genetically resistant mice ($Fv\text{-}2^{rr}$), adaptation occurs resulting in an alteration of the SFFV env protein produced, followed by development of erythroleukemia in adult animals. The adapted viruses in this study are the RB and BSB. To determine if this disease is similar to that induced with the wild-type FV, pathology of the infected spleens were compared. Mice were infected with the various viruses and at nine days the mice were terminated, and the spleens were weighed, fixed, examined for foci, and prepared for histologic examination. A unique morphology was seen in the spleens infected with the adapted viruses which was distinct from the wild-type disease as previously reported. The significance of this morphology of the spleen is under further investigation. Observing alterations in the pathology of the erythroleukemia produced by the adapted viruses may provide some insight into the target cell(s) and factors resulting in cell transformation and mechanism of $FV\text{-}2^{rr}$ resistance.

OP2.3

INTERMEDIATE FILAMENTS EXPRESSION IN NORMAL AND TRANSFORMED CELLS. J.A. Evanson, G. Merkel, Y.C. Chen. Department of Biological Sciences, Indiana University-Purdue University at Fort Wayne, Fort Wayne, IN 46805-1499, USA.

We have been studying the changes of the expression of intermediate filament protein, vimentin in rat kidney cells transformed by a temperature-sensitive mutant of avian sarcoma virus. These cells exhibit the round transformed phenotype at the permissive temperature (33°C) and revert to normal fibroblast phenotype at the nonpermissive temperature (38°C). By use of monoclonal anti-vimentin antibodies, we have revealed different patterns of vimentin filaments in normal and transformed cells. At the nonpermissive temperature, vimentin filaments are evenly distributed throughout the cell cytoplasm, whereas at permissive temperature, they appear to be concentrated at peripheral surface of the cell membrane. These results indicate that malignant transformation of mesenchymal type of cells in vitro correlates significantly to the changes in vimentin expression.

OP2.4

INFLUENZA VIRUS HEMAGGLUTININ DISTINGUISHES SIALYLOLIGOSACCHARIDES IN MEMBRANE-ASSOCIATED GANGLIOSIDES AS ITS RECEPTOR THAT MEDIATES THE ADSORPTION AND FUSION PROCESS OF VIRUS INFECTION Yasuo Suzuki, Yasuhiro Nagao, Hideshige Kato, Makoto Matsumoto, Kuniaki Nerome¶, Katsuhisa Nakajima§, Eri Nobusawa§ Department of Biochemistry, Shizuoka College of Pharmacy, Shizuoka-shi 422, Japan, ¶National Institute of Health, Shinagawa-ku, Tokyo 141, Japan, §The Institute of Health Science, University of Tokyo, Shinagawa-ku, Tokyo 108, Japan.

Human influenza A and B viruses bearing antigenically different hemagglutinin serotypes caused extensive hemagglutination, low pH fusion and hemolysis of asialoerythrocytes reconstituted with gangliosides. Sialylparaglobosides, I-active and i-active gangliosides, GM3 and GM1b commonly exhibited significant receptor activity toward the viruses. A/PR (H1) recognized sialylparagloboside containing NeuAcα2-3Gal sequence preferentially, whereas A/Japan (H2) and A/Aichi (H3) recognized the NeuAcα2-6Gal preferentially over NeuAcα2-3Gal. B/Lee recognized both NeuAcα2-3Gal and NeuAcα2-6Gal sequences most equally. Responsiveness of erythrocytes labeled with gangliosides containing NeuGc to the viruses was considerably lower than that of erythrocytes containing gangliosides with NeuAc. The activities of GM1a, GM2 and GD1b bearing NeuAc on inner galactose of ganglio-series core were also very low. Above results indicate that human influenza viruses recognize the oligosaccharides of gangliosides as its receptor and differentiate the microdomains of oligosaccharides such as sialic acid species and the sialic acid-Gal linkages.

OP2.5

DIFFERENTIAL PRODUCTION OF ENDOGENOUS PYROGEN (EP) BY HUMAN LEUCOCYTES INTERACTED WITH H1N1 AND H3N2 INFLUENZA VIRUSES. C.M. Tinsley, D.M. Coates, C. Sweet, H. Smith. Department of Microbiology, University of Birmingham, Birmingham, United Kingdom.

Fever and the other constitutional effects of influenza (eg. headache, myalgia, anorexia, depression) result from the liberation of EP from phagocytes. These effects have been reported to be milder for recent H1N1 influenza viruses than for strains of the H3N2 serotype. Interaction between influenza viruses and human peripheral blood leucocytes in vitro showed that H1N1 strains, A/USSR/90/77 and A/Fiji/15899/83, elicited significantly less EP than two virulent clones, 7a and 64c, of the A/Puerto Rico/8/34-A/England/939/69 (H3N2) reassortant virus system. Similar observations were made with UV-inactivated stocks of A/Fiji/15899/83 and clone 64c. These results, which are in accord with the differential severity of fever produced by these strains in both intranasally infected and intracardially inoculated ferrets, show that influenza virus strains may differ in their capacity to induce EP from phagocytes. Furthermore, the observations with inactivated virus suggest that certain virion components are pyrogenic and may differ in quantity or nature between strains. These results are important in relation to the differential severity of influenza epidemics and the reactogenicity of vaccine strains.

OP2.6

INSERTIONAL ACTIVATION OF c-myb BY REV IN LYMPHOBLASTIC LEUKEMIA OF MUSCOVY DUCKS
A. Gazit[1], M. Malkinson[2] and A. Yaniv[1]
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Two spontaneous outbreaks of an undifferentiated lymphoblastic leukemia occurred in breeding flocks of Muscovy ducks. Both immunological surveillance and a search for mature retrovirus particles in the blood plasma and tumor cells failed to provide any clue of a viral etiology. However, nucleic acid hybridization studies demonstrated that reticuloendotheliosis virus (REV)-related nucleic acid sequences were integrated de novo and specifically expressed in the tumor cells. Southern analysis of tumorous DNA revealed that the newly inserted REV-related sequences do not contain 'rel' sequences, indicating that the helper REV-A virus is implicated in this malignancy. Hybridization to various oncogenes revealed rearrangement of the proto-oncogene c-myb. Subsequently we demonstrated that this rearrangement was due to the integration of REV-like sequences into the Muscovy duck c-myb locus. We suggest that insertional mutagenesis mechanism was involved in the malignant activation of the proto-oncogene c-myb, which led to the induction of the undifferentiated lymphoblastic leukemia in Muscovy ducks.

OP3.1

NUCLEOTIDE SEQUENCE OF THE M RNA SEGMENT OF UUKUNIEMI VIRUS
R. Rönnholm and R.F. Pettersson. Ludwig Institute for Cancer Research, Stockholm, Sweden.

Uukuniemi (UUK) virus and other bunyaviruses mature in the Golgi complex (GC). The membrane proteins G1 (M_r 75,000) and G2 (M_r 65,000) which are encoded by the M RNA segment, are transported to and accumulate in the GC. This seems to be one important factor determining the site of the virus maturation. Are there specific signals that guide the membrane proteins to the GC and make them stay there? As a first step to answer this question we have determined the primary sequence of G1 & G2 from cDNA copies of the virion RNA segment. The RNA segment is 3231 residues long (Mr 1.1×10^6). A single open reading frame comprising 1008 amino acids was found in the mRNA-sense strand between nucleotides 18-3042 corresponding to the 110,000-dalton precursor (p110) of G1 & G2. Both G1 & G2 are preceeded by a stretch of 17 hydrophobic amino acids likely to represent the signal sequences. G1 & G2 are anchored to the lipid bilayer by 19 and 27 hydrophobic amino acids, respectively. A weak sequence homology between the UUK virus and Phlebovirus M RNA products was found. Attempts to express G1 & G2 in animal cells are under-way.

OP3.2

THE PROTECTION OF SHEEP BY INOCULATION WITH A LIVE ATTENUATED DERIVATIVE OF RIFT VALLEY FEVER VIRUS.
K A Hubbard*, A Baskerville+ and J R Stephenson*. Vaccine Research and Development Laboratory* and Experimental Pathology Laboratory+, CAHN, Porton Down Salisbury, UK.

Rift Valley Fever (RVF) is a disease of economical and clinical importance in many regions of Africa. Although vaccines against the disease are available, they are either too expensive or too abortogenic to use on a scale sufficient to effectively control the disease. A varient of RVF has been developed at USAMRIID, Fort Detrick, USA, by repeated passages in the presence of a mutagen and has been shown to have a reduced pathogenicity for small rodent. The pathogenicity of this varient has been tested in young lambs and no clinical or histopathological signs of disease were observed. Doses as low as 100 p.f.u. per animal were shown to be protective against challenge with virulent virus with little or no observable tissue damage.

OP3.3

ISOLATION OF A HANTAAN-RELATED VIRUS FROM MUS MUSCULUS IN THE UNITED STATES.
L.J. Baek, R. Yanagihara, C.J. Gibbs, Jr. and D.C. Gajdusek, National Institutes of Health, Bethesda, Md., U.S.A.

A Hantaan-related virus was isolated from a Mus musculus captured in August, 1986, in Real County, Texas. Virus-specific fluorescence was first detected 13 days after inoculation of Vero E6 cells with lung and spleen tissues from the seropositive Mus. Using negative-stain electron microscopy, the new isolate, designated Leakey virus, resembled other hantaviruses, with round and long forms. Leakey virus induced a fatal meningoencephalitis in infant Fischer rats, with viral antigen detectable in brain, lung, liver, kidney and spleen. Extensive cross-reactivity was found between Leakey virus and Hantaan, Seoul, Puumala and Prospect Hill viruses, using the indirect immunofluorescent antibody technique. However, preliminary results of serum-dilution, plaque-reduction neutralization tests revealed no cross-neutralization. Our data indicate that another serotype of hantavirus may be enzootic among murid rodents in the United States. Studies to further characterize this virus and to determine its role in human infection are underway.

OP3.4

EXPRESSION OF PICHINDE VIRUS POLYPEPTIDES USING RECOMBINANT VACCINIA VIRUS TO TEST FOR RECOGNITION AND LYSIS BY VIRUS-SPECIFIC H-2 RESTRICTED CTL.
D.Y. Ozols, K.L. Rosenthal, W.E. Rawls, and D.G. Harnish. Molecular Virology and Immunology Program, Department of Pathology, McMaster University, Hamilton, Ontario, Canada. L8N 3Z5

Intravenous infection of adult mice with Pichinde virus elicits a strong cell-mediated immune response that includes virus-specific H-2 restricted CTL. We have shown that CTL restriction maps to the K region of $H\text{-}2^b$. Pichinde virus, a member of the Arenaviridae, has a single-stranded RNA genome of two segments, S and L. The S RNA contains sequence information for the nucleoprotein, and glycoprotein precursor, which is post-translationally cleaved into GP1 and GP2. We have cloned cDNA corresponding to Pichinde virus strain AN3739 RNA into λgt11. By screening the library with hamster anti-Pichinde sera, we obtained clones expressing immunologically detectable fusion proteins of the entire nucleoprotein gene . Coding sequences for the N-terminal 192 of 276 amino acids of GP1 were obtained using an oligonucleotide probe. cDNAs were subcloned by recombination into vaccinia virus. Production of Pichinde virus-specific products, in recombinant vaccinia infected cells, has been assessed. These cells will be used to determine the viral polypeptides required for CTL recognition and lysis.

Supported by the Medical Research Council of Canada.
D.Ozols holds an NSERC post-graduate scholarship.

OP3.5

ANALYSIS OF THE S-TRANSCRIPTIONAL COMPLEX FROM AKABANE VIRUS-INFECTED CELLS.
G. Abraham. School of Science, Griffith University, Nathan, Queensland, Australia.

Little information is available concerning the nature of the mRNAs coded by the S gene of bunyaviruses. This gene has the coding potential for more than one type of mRNA and genetic and molecular evidence has indicated that two viral proteins found in infected cells have their coding origins in the S gene.

This report analyses the nature of the S-gene transcriptional complex found in Akabane virus-infected cells by terminal RNA labelling procedures. Accessible 5'terminii were labelled with or without prior dephosphorylation using polynucleotide kinase. The products were analysed by gel electrophoresis in either denaturing or non-denaturing conditions. Similar results were obtained when 3'-end labelling procedures were used. However, when the M gene transcriptional complex was analysed only one such product could be detected. An analysis of these results will be presented.

OP3.6

TACARIBE COMPLEX VIRUSES; EVIDENCE FOR TWO ANTIGENIC SUBGROUPS.
A. Wozniak, A. Sanchez, J.B. McCormick, and M.P. Kiley. Special Pathogens Branch, Division of Viral Diseases, Centers for Disease Control, Atlanta, GA 30333, USA.

The antigenic relationships between nine "New World" arenaviruses were assessed by the immunofluorescent antibody test (IFAT), the enzyme-linked immunosorbent assay (ELISA), and the plaque reduction neutralization test (PRNT) The tests were performed with infected cell antigen using polyclonal hamster sera and monoclonal antibodies raised to five of the nine viruses (Amapari, Junin, Machupo, Parana, and Tacaribe). Two major antigenic groups were identified by analysis of the IFAT and ELISA antibody titers by the Pearson product moment correlation statistic. The first or Tacaribe group contains Tacaribe, Amapari, Junin, Latino, and Machupo viruses. The second or Parana group contains Parana, Flexal, Pichinde, and Tamiami viruses. The monoclonal antibodies to nucleoprotein, as determined by radioimmunoprecipitation, showed both group and subgroup specificity.

OP3.7

STUDIES ON THE EXPRESSION OF VACCINIA VIRUS CONTAINING THE M AND S RNA SEGMENTS OF HANTAAN VIRUS. M. Pensiero, G.B. Jennings, J. Dalrymple, K.V. Holmes, J. May. Department of Microbiology, Uniformed Services University of the Health Sciences, Bethesda, U.S.A.

We have cloned the M and S RNA segments of Hantaan virus into vaccinia virus. The recombinant vaccinia viruses were used in in vivo and in vitro studies.

We have shown by immune precipitation that the vaccinia recombinant containing the entire M segment, G-Vac, expresses both G1 and G2 in infected cells. Vaccinia virus containing the S segment, N-Vac, produces the nucleocapsid protein. Immunofluorescence using monoclonal antibodies against G1 and G2 show exclusive Golgi association in G-Vac infected cells. In contrast, N-Vac infected cells show the nucleocapsid protein in discrete complexes throughout the cytoplasm.

Tail scarification of BALB/CyJ mice inoculated with G-Vac raised neutralizing antibodies against Hantaan virus. N-Vac inoculated mice raised antibodies to Hantaan virus as demonstrated by RIA but did not elicit a neutralizing antibody response.

We have constructed several vaccinia recombinants containing deleted portions of the M segment. Their expression in terms of IF and specific immune response has been compared to that of G-Vac.

OP3.8

HEMORRHAGIC FEVER WITH RENAL SYNDROME IN WESTERN GERMANY: SEROEPIDEMIOLOGICAL STUDIES IN HUMANS AND RODENTS.

J. Pilaski, R. Peceny, O. Gorschewsky, L. Zöller, V. Kraft, H.W. Lee. Medical Institute of Environmental Hygiene, D-4000 Düsseldorf, Federal Republic of Germany.

Seroepidemiological studies of hemorrhagic fever with renal syndrome (HFRS) virus infection in humans and also in different species of wild-living and laboratory-bred rodents were carried out in Western Germany in order to elucidate the existence of endemic foci of hantavirus. The indirect immunofluorescent antibody (IFA) technique was applied using Vero-E6 cells infected with different hantavirus strains (76-118, NE-Hällnäs, SR-11). A seroepidemiological survey in 3 hospitals at Düsseldorf revealed that 10 out of 820 (1.2 %) in-door patients have antibodies against hantavirus. In 1985 a total of 3 clinical cases with severe renal failure was observed in humans living in the southern part of Germany, all being confirmed by serological examination. Hantavirus antibodies were also found in 3 free-living rodent species, i.e. Rattus norvegicus, Clethrionomys glareolus and Microtus arvalis, and in different breeding stocks of laboratory rats. One Clethrionomys glareolus trapped in August 1986 in the surrounding of the living place of one clinical patient turned out to be serologically positive.

OP3.9

USE OF HETEROLOGOUS ANTIBODY MOLECULES AND $F(ab')_2$ PORTIONS FOR PROTECTING GUINEA PIGS (GP) INFECTED WITH JUNÍN VIRUS (JV). R. Kenyon, D. Pifat, P. Jahrling, R. Condie, J. Maiztegui, D. Brantley, and C.J. Peters. USAMRIID, Ft. Detrick, Frederick, MD 21701-5011

Convalescent, whole, human plasma is of proven value in treatment of Argentine hemorrhagic fever (AHF). Because safety and uniformity of such treatment are contentious, we investigated alternate means of passive immunization. Using a GP model for AHF, we determined protective efficacy of: 1. immunoglobulin fractions from AHF-immune humans; and 2. mouse monoclonal antibodies (MAb). The $IgG_{1,2,3,4}$ and the $IgG_{1,2,3,4}$ $F(ab')_2$ protected infected GP. While the $F(ab')_2$ neutralized viral plaques in cell culture, it neither protected nor increased time-to-death of GP given even 5 times as much antibody, based on plaque reduction neutralization (PRN) titers. Mouse MAb also protected GP. The MAb were generated against nucleocapsid or the 38,000 MW glycoprotein, and only the latter protected. Those MAb capable of protecting infected GP were those capable of complement-mediated cytolysis, regardless of the PRN titers. AHF may be an ideal model system for testing protective efficacy of mouse-human MAb. Our data suggest that elimination of infected cells may be the critical event in protection, rather than neutralization of JV. Cell transfer experiments support this. When B and T spleen cell-enriched populations from immune GP were prepared by soybean agglutinin-L, the T cell-enriched population neither mediated virus-specific cytolysis in vitro nor protected. However, the B cell-enriched population both lysed and protected.

OP3.10

MOLECULAR CLONING OF JUNIN VIRUS RNA.
P.D.Ghiringhelli, R.Rivera, M.Baro, O.Grau and V.Romanowski. Department of Biochemistry, School of Sciences, University of La Plata, 1900 - La Plata, Argentina.

Junín virus is one of the few arenaviruses pathogenic to humans. In order to study some aspects of the molecular biology of this virus and examine the possibility of using recombinant DNA technology to produce new diagnostic reagents and vaccines we have constructed molecular clones of the Junín virus genome. A synthetic oligonucleotide complementary to the 3'ends of arenavirus L and S RNAs was used to prime cDNA synthesis on Junín virus total RNA isolated from purified virions. Double stranded cDNA was cloned into the HincII or the EcoRI site of the plasmid pUC18. Clones were screened by their ability to hybridize to short copy cDNA or to synthetic oligonucleotides. They were also characterized by restriction mapping and Northern blot analysis. Clones bearing Junín virus L and S RNA sequences were obtained, but only S cDNA clones were further analyzed, and the nucleotide sequence of the nucleocapsid protein gene (N) was determined. The first AUG codon (nucleotides 82-84 from the 5'end of the viral complementary RNA) initiates a long open reading frame encoding the N protein. The predicted aminoacid sequence was compared to the homologous gene products of other arenaviruses, and a high degree of identity (70%) was observed between Junín and Tacaribe viruses. Much lower percentages of identical sequences were apparent from comparisons of Junín virus N protein with those of Pichinde, Lassa and LCM.

OP3.11

GENETIC REASSORTMENT BETWEEN JUNIN AND LCM ARENAVIRUSES.
V.Romanowski, M.I.Baro, P.G.Remorini and O.Grau. Department of Biochemistry, School of Sciences, University of La Plata, 1900 - La Plata, Argentina.

Junín (JUN) virus is the etiologic agent of Argentine hemorrhagic fever (AHF). Another arenavirus, lymphocytic choriomeningitis (LCM) virus, has also been isolated from rodents and human patients in the AHF endemic area.

In the present study we address the question of whether JUN and LCM viruses are able to exchange their genetic information by means of reassortment of their L and S RNA segments. To test this hypothesis BHK21 cells were coinfected with pairs of JUN and LCM *ts* mutants, and the *wt* progeny was detected by its ability to produce plaques at the non-permissive temperature (40°). Two JUN *ts* mutants yielded *wt* progeny at frequencies of 1-6% when coinfections were performed with LCM(WE strain) mutants bearing *ts* lesions in the S RNA. These genetic experiments suggested, therefore, that reassortment between JUN and LCM viruses occurs and that the (L/S) genotype of the *wt* reassortant viruses should be (LCM/JUN). To confirm these data, RNA extracted from the parental *ts* viruses and plaque-cloned reassortants was subjected to dot-blot hybridization analyses using probes derived from LCM L and S cDNA clones.

We conclude from these results that, under appropriate conditions, genetic exchange between JUN and LCM viruses is possible. However, the question of whether genetic reassortment is significant in nature remains open.

OP3.12

ARENAVIRUSES IN SOUTHERN AFRICAN RODENTS. R Swanepoel, P.A. Leman and A.J. Shepherd, National Institute for Virology, Sandringham 2131, Republic of South Africa.

Sera of 4023 rodents collected over a period of 16 years in South Africa and Zimbabwe for unrelated purposes, were tested by indirect immunofluorescence (IF) for antibody to African arenaviruses using cells infected with Lassa fever and Mopeia viruses as antigen. Antibody was found in 15/28 species; most frequently in Aethomys namaquensis [20/136 (14.7%) positive; titers 1:8 - 2048], Mastomys natalensis (2N=32)[311/637 (48.8%) positive; titers 1:32 - 16384], Otomys irroratus [46/213 (21.6%) positive; titers 1:8 - 8192] and Otomys unisulcatus [33/164 (20.1%) positive; titers 1:8 - 2048]. In contrast, only 6/620 (1.0%) Mastomys couche (2N=36) had antibody. 832 serum and organ specimens from 713 individuals of 4 species were tested in cell cultures and 17 arenavirus isolations were obtained: 10 from M.natalensis, 5 from O.unisulcatus and 1 each from O.irroratus and A.namaquensis. Preliminary characterisation, including tests with monoclonal antibodies, suggest that the M.natalensis viruses correspond to Mopeia virus while the other isolations appear to comprise two new arenaviruses.

The medical significance of the arenaviruses in southern Africa has yet to be determined.

OP3.13

ANALYSIS OF EPIDEMIC HEMORRHAGIC FEVER PATIENTS SERUM PROTEINS IN POLYACRYLAMIDE GEL ELECTROPHORESIS. Zhang Tian-ming, Huiang Chin-min. Virus Research Institute, Hubei Medical College, Wuchang, Hubei, PRC

Analysis of epidemic hemorrhagic fever (EHF) patient's serum proteins inPAGE is reported. The PAGE maps showed to difference between EHF patient's and normal human serum proteins. In paired sera of EHF patients, the electrophoresis maps showed a difference between serum protein in acute phase and in convalescents phase. In convalescent-phase sera of ehf patients, there were also some difference. This would mean complexity of EHF patient's serum protein could be shown by PAGE method.

OP4.1

ANTIVIRAL EFFECT OF ACYCLOVIR AND INTERFERON AGAINST HSV-1 IN VARIOUS HUMAN NEURAL CELLS. N.Hanada, M.M. Rahman, S. Kido, M. Terashima, M. Miyazu, T. Morishima. Department of Pediatrics, Nagoya University School of Medicine, Nagoya, Japan.

Despite the development of antiviral agents for herpes virus infections, the progress of the disease could not be prevented in some cases of herpes encephalitis. We tried to determine whether acyclovir (ACV) was actually effective in inhibiting the virus growth in various neural cells and if other antiviral agent such as interferon- α(IFN) could prevent the virus growth alone or in combination with ACV in those cells. Human neuroblastoma (IMR), glioblastoma (MGC) and glioma (U251MG) cell lines were infected with HF strain of HSV-1 in the presense of ACV and/or IFN. Yields were determined 48-72 hours after inoculation. Permissive growth of HSV-1 was demonstrated in each cell line without any treatment. In IMR, ACV remarkably reduced the virus yields, while IFN was not effective. In contrast, IFN significantly inhibited the virus growth in MGC, but ACV did not. In U251MG, both ACV and IFN suppressed the virus growth. The combination effects of ACV with IFN were demonstrated in all cell lines. These results were confirmed by immunofluorescence. Thus, antiviral activities of ACV or IFN could depend on the kind or nature of the neural cells. Therefore, the combination therapy might prevent the virus spread more effectively in central nervous system of herpes encephalitis patients.

OP4.2

EFFECTS OF RIBAVIRIN ON HSV-2 SYSTEMIC MURINE INFECTION IN PRESENCE OF A SPECIFIC ADOPTIVE TRANSFER. A. Arena , L. Bonina, M.C. Liberto, R.A. Marendino, P. Mastroeni. Microbiology Institute, Faculty of Medicine, Messina University, Messina, Italy.

Several attempts have been made in the chemotherapy field in order to eradicate herpetic infections. The present study was undertook to analyse the possibility of cooperation between chemotherapy and immunotherapy in hindering HSV infections. For this purpose Ribavirin was used as antiviral agent and the imunotherapy was carried out by a specific adoptive transfer of syngeneic Antigen Presenting Cells (APC). The experimental model used consists of a murine herpetic infection in the liver caused by HSV type 2 strain Bry. The results obtained demonstrate: an high degree of protection after the adoptive transfer showed in terms of survival, reduction of viral multiplication in the liver as well as the rise of a specific immune response; positive relationships between Ribavirin and adoptive transfers showed by a better degree of protection. These findings could be interpretated by a favourable action of Ribavirin during the immune response versus HSV.

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OP4.3

ANTIVIRAL EFFECTS OF GLYCYRRHIZIN ON VARICELLA-ZOSTER VIRUS AND HUMAN IMMUNODEFICIENCY VIRUS *in vitro*. S. Shigeta, M. Ito[1], M. Baba, E. De Clercq, R. Pauwels[2], H. Nakashima and N. Yamamoto[3].
Department of Bacteriology, Fukushima Medical College, Fukushima, Japan[1] Rega Institute, Catholic University of Leuven, Leuven, Belgium[2], Department of Virology and Parasitology, Yamaguchi University School of Medicine, Ube, Japan[3]

Glycyrrhizin (GL) is an aqueous extract of licorice root (Glycyrrhiza Radix) which is known as an anti-inflammatory substance in Chinese Medicine.
GL showed antiviral activity against varicella-zoster virus (VZV) and human immunodeficiency virus (HIV, HTLV-III) *in vitro*. The average 50% inhibitory dose (ID_{50}) for plaque formation by 5 VZV strains in human embryonic cells was 0.7 mM the ID_{50} of GL for plaque formation by HIV in MT-4 cells was 0.15 mM. The selectivity indexes (ratio of ID_{50} for host cell DNA synthesis to ID_{50} for virus replication) were 30 for VZV and 17.3 for HIV. GL had no direct effect on the DNA polymerase of VZV and the reverse transcriptase of HIV. We postulate that GL inhibits the cell-to-cell spread of VZV; however, the mechanism of anti-HIV action remains to be elucidated.

OP4.4

BIOLOGICAL PROPERTIES OF AMANTADINE-RESISTANT INFLUENZA VIRUS MUTANTS. W. J. Bean, R. G. Webster. Department of Virology and Molecular Biology, St. Jude Children's Research Hospital, Memphis, Tennessee, USA.

The highly virulent avian influenza virus Chicken/Penn/1370/83 has been used as a model system for the study of the generation and biological properties of amantadine-resistant influenza virus mutants.

Chickens infected with this virus and given the drug in their water supply are protected from lethal disease, but continue to shed virus from their respiratory tract and in their feces. Some of this virus is amantadine-resistant and birds infected by direct contact with the infected birds develop a lethal infection despite the presence of the drug. Amantadine-resistant virus strains isolated from these contact birds and those selected in cell culture have been characterized and compared with the wild-type virus. The drug-resistant isolates vary in virulence, with some being similar to the wild-type and some being attenuated, but no consistent differences in growth characteristics have been observed. Reversion to wild-type has not been observed after passage in the absence of the drug. No mutant has been found with an altered pH for membrane fusion, and all of the amantadine-resistant isolates have mutations in the M2 polypeptide.

OP4.5

ANTIVIRAL EFFECTS OF 9-(TRANS-2', TRANS-3'-DIHYDROXYCYCLOPENT-4'-ENYL)-ADENINE (1) AND -3-DEAZAADENINE (2). M. Hasobe, J. McKee, D. Borcherding, R. T. Borchardt. Departments of Pharmaceutical Chemistry and Medicinal Chemistry, University of Kansas, Lawrence, Kansas, U. S. A.

Analogs 1 and 2 of neplanocin A (NpcA), lacking NpcA's 4'-hydroxymethyl group and differing structurally in the adenine base of 1 and the 3-deazaadenine base of 2, were designed to optimize antiviral activity by retaining NpcA's inhibitory effects against S-adenosylhomocysteine (AdoHcy) hydrolase and to minimize cytotoxicity by reducing NpcA's substrate activity for adenosine kinase. The antiviral activities of NpcA, 1 and 2, expressed as IC_{50} values (concentrations needed to inhibit 50% of vaccinia virus plaque formation), are 0.08 μM, 0.28 μM and 0.95 μM, respectively. The cytotoxicities, determined by measuring the effects of the test compounds on L929 cell viability and expressed as ID_{50} values (concentrations needed to suppress 50% of cell growth over 72 hours), of NpcA, 1 and 2 are 0.5 μM, 17 μM and 58 μM, respectively. The antiviral effectiveness values (ID_{50}/IC_{50}) for Npc A, 1 and 2 are 6, 61 and 59, respectively. When cytotoxicity was measured as inhibition of cellular RNA and DNA synthesis, analogs 1 and 2 were shown to have no effects on DNA or RNA synthesis, but NpcA selectivaly inhibited RNA synthesis. In separate studies, 1 and 2 have been shown to be potent inhibitors of L-cell AdoHcy hydrolase but not substrates for cellular adenosine kinase. These results further confirm that AdoHcy hydrolase is the molecular target which mediates the antiviral effects and that adenosine kinase transformation of neplanocin A may mediate its cytotoxic effects. (Supported by NIGMS grant #GM-29332)

OP4.6

ANTIVIRAL SUBSTANCE FROM PHOTOTROPHIC BACTERIA

M.Kobayashi, H.Hirotani and E.Takahashi.

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A phototrophic bacteria, Rhodopseudomonas capsulata, was found to produce antiviral substance. This substance was purified and has shown to inactivate bacteriophage T5 and several pathogenic viruses such as poliovirus, sindbis virus, etc., when it was kept in contact with the viruses at the concentration of 0.1ug/ml. The antiviral effect of the substance was enhanced when Cu^{2+} or ascorbic acid was present and was inhibited when Fe^{2+} was present. It is also noteworthy that this substance showed no toxicity to the host cells. We will also report the result from our research on its chemical structure.

It was also found that several fresh vegetables were producing antiviral substance similar to that of the phototrophic bacteria.

OP5.1

MAPPING TMV SEQUENCES INVOLVED IN VIRAL-INDUCED HYPERSENSITIVITY. D.A. Knorr, W. O. Dawson. Plant Pathology Department, University of California, Riverside, California 92521, USA.

Sequences of tobacco mosaic virus involved in causing hypersensitivity in tobacco plants carrying the N' gene are being mapped. Mutants causing local lesions in Nicotiana sylvestris were derived from a wild-type population produced from inoculum generated by in vitro transcription of plasmid pTMV204, which contains a full-length insert of cDNA made to TMV strain U1. TMV204-D1, a local lesion mutant obtained after nitrous acid treatment of the parent strain (TMV204), was completely mapped. A series of artificial recombinants was constructed in which fragments from cloned cDNA to TMV204-D1 replaced the corresponding sequence in pTMV204. Constructs were screened for ability of in vitro RNA transcripts to produce local lesions in N. sylvestris. It was determined that sequences neccesary to convert pTMV204 to the mutant phenotype were present within a 256 bp BsmI - MaiI fragment (nucleotides 5972-6206). Sequence analysis of this fragment showed two nucleotide changes. At base 6157, within the coat protein open reading frame, a C to U substitutes phenylalanine for serine at residue 147. In the 3' non-translated region, nucleotide 6199 has been changed from A to G. Other local lesion mutants are currently being examined for differences by similar methods and by direct RNA sequencing.

OP5.2

MOLECULAR STUDIES OF POTATO LEAFROLL VIRUS M. Mayo, D. Robinson, P. Massalski. Scottish Crop Research Institute, Invergowrie, Dundee, U.K.

The organization and expression of the genome of potato leafroll luteovirus (PLRV) is being studied by translation, by antigenic analysis with monoclonal antibodies to the main translation product, the coat protein (Mr 24500), and by nucleotide sequencing.

In wheat germ extracts PLRV RNA directs the synthesis mainly of Mr 70000 and Mr 28000 polypeptides; the largest product is about Mr 120000. Little or no coat protein was detected in vitro but it is the major virus-specific protein made in infected protoplasts. Northern blot analysis of RNA from infected leaves or protoplasts revealed a major subgenomic RNA of 3.4 kb not found in virus particles. This may be a coat protein mRNA.

Monoclonal antibodies specific for two distinct conformation-insensitive epitopes reacted in immunoblotting experiments with a cyanogen bromide fragment of coat protein of about Mr 13000. Thus at least two epitopes are present in one half of the coat protein molecule.

Complementary DNA copies of artificially polyadenylated PLRV RNA have been cloned in plasmid vectors and results of sequencing studies will be presented.

OP5.3

MULTIPLICATION, PURIFICATION AND SEROLOGY OF RICE TUNGRO BACILLIFORM AND SPHERICAL VIRUSES. H. Hibino and P.Q. Cabauatan. International Rice Research Institute, Los Banos, Philippines.

Rice tungro bacilliform virus (RTBV) depends on rice tungro spherical virus (RTSV) for its transmission by the leafhopper Nephotettix virescens. To multiply RTBV infected rice plants, leafhoppers that had fed on plants infected with both RTBV and RTSV were given serial access to anti-RTSV serum through membrane for 16 h, to RTBV-infected plants for 8 h, and then to rice seedlings for a 1-d inoculation feeding. Inoculated seedlings were mostly infected with RTBV alone. Seedlings infected with RTBV alone were selected and used for RTBV purification. A RTSV-infected plant was selected from plants which were inoculated using leafhoppers fed on RTBV/RTSV-infected plants. The RTSV isolate was multiplied in rice seedlings and purified.

$A_{260/280}$ of purified RTBV and RTSV fractions ranged 1.13-1.20 and 1.52-1.75, respectively. Purified RTSV was about 31 nm in diameter and RTBV was about 35 nm in width and 110-310 nm in length.

Rabbit antisera to RTBV and RTSV were obtained: the titers were 1/640 and 1/2560, respectively. Latex test and ELISA specifically detected RTBV and RTSV in leaf extracts.

OP5.4

CHARACTERIZATION OF INFECTIOUS RNA OF AN UNKNOWN VIRUS ISOLATED FROM MEDICAGO SATIVA. L. Beczner, R.I. Hamilton, Gy. Bisztray. Plant Protection Institute, Hungarian Academy of Sciences, Budapest, Hungary, Agriculture Canada, Research Station, Vancouver, Canada.

Together with alfalfa mosaic virus an unknown virus was isolated from alfalfa showing mosaic. It was detectable neither by serology and electronmicroscopy nor by classical methods of virus purification. From the leaves of infected plants dsRNA-s of the unknown virus with molecular weights of $5x10^6$, $4.2x10^6$ and $3.6x10^6$ were detected. The virus is separable from AlMV on hosts, and can be maintained in single culture. In complex it reduces the multiplication of AlMV and changes the ratio of its genomic RNA-s. The host range of the infectious RNA is narrow: i.e. it locally and systemically infects Phaseolus vulgaris, Vigna sinensis, Chenopodium amaranticolor, C. murale, C. quinoa, Nicotiana benthamiana, N. clevelandii, N. megalosiphon, Tetragonia expansa. Mechanically easily transmissible. It is seed-transmissible by C. murale /cca. 30 %/. The naked RNA retains infectivity for years at -20°C in leaves as well as in freeze-dried form. The relationships of the unknown virus to AlMV and other viruses is discussed in the poster.

OP5.5

NEW STRAINS OF THE PEANUT STUNT VIRUS ISOLATED FROM LEGUMES IN POLAND. H. Pospieszny. Department of Plant Virology, Institute for Plant Protection, Poznań, Poland.

Two strains of the peanut stunt virus /PSV/, designated as PSV-P and PSV-G, have been isolated from legumes. The both PSV strains are very pathogenic to legumes and differ by the type of infection on some plants, first of all by symptoms.

PSV-G contrary to PSV-P strongly reduces growth of infected plants and causes the death of bean and lupin apices. PSV-G is very stable and attains a several-fold larger concentration in plants than does PSV-P. Serological studies showed that the both PSV strains, besides common antigenic determinants, have also determinants specific to them. The PSV-G genome is four-parted, and in the PSV-P genome there additionally occurs RNA-5.

Three is a large genetic diversity within in Poland, since besides two strains from legumes we have managed to isolate the third one from selery. A large genetic diversity and high pathogenicity make the PSV a potentially dangerous pathogen of legumes in Poland.

OP5.6

CHARACTERIZATION OF INTERACTION BETWEEN TOMATO TOLERANCE GENE Tm-1 AND TMV. Y.Watanabe, T.Meshi, N.Kishibayashi, A.Adachi, F.Motoyoshi, Y.Okada. Department of Biophysics and Biochemistry, Faculty of Science, The University of Tokyo, Hongo, Tokyo 113, JAPAN.

Tm-1 is known as a gene which confers tomato plants resistance to tobacco mosaic virus. We established three species of suspension cultures from Tm-1/Tm-1, Tm-1/+ (F1 plant between Tm-1/Tm-1 and +/+ plant), +/+ tomatoes and then a novel infection system with a small amount of TMV RNA by electroporation. Recently, one of the authors has isolated an "overcomer" mutant, which can multiply in tomato plants homozygous for Tm-1 (Tm-1/Tm-1), from tomato strain L. The mutant was designated as Ltal. Protoplasts prepared from +/+ tomato sustained the multiplication of both L and Ltal strains, while those from Tm-1/+ and Tm-1/Tm-1 did resist being infected by L but not by Ltal strain. Viral proteins (130K, 180K, 30K and coat proteins) and RNAs (genomic RNA, 30K protein mRNA, coat protein mRNA and replicative form RNA) were synthesized in an "all-or-none" fashion depending on the combination of host genotype and virus. In parallel, the genomic sequence of Ltal was determined by chain termination method and compared with that of wild type L. The result revealed amino acid changes in the 130K (180K) protein cistron and suggested that a host factor encoded by Tm-1 gene interacts directly with the putative viral replicase gene product.

OP5.7

COAT PROTEIN GENE ENCODES A HOST RESPONSE DETERMINANT.
T. Saito, T. Meshi, N. Takamatsu, Y. Okada.
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Plants with the N' gene such as Nicotiana sylvestris respond differently to infections of two closely related strains of tobacco mosaic virus (TMV), OM (common strain) and L (tomato strain). TMV-OM spreads systemically with mosaic symptoms, while TMV-L induces the necrotic response, causing local lesions in the N' plants. To reveal the viral factor of TMV-L inducing the host necrotic response, we have constructed several recombinant viruses between the two strains by in vitro transcription from the recombinant cDNAs, in which the cDNA of TMV-L RNA was partly replaced by that of TMV-OM. The recombinant viruses having the coat protein gene of TMV-OM in place of that of TMV-L produced no necrotic local lesions but spread systemically with mosaic symptoms, while the others having the coat protein gene of TMV-L produced necrotic local lesions. These observations indicate that the viral factor of TMV-L responsible for the necrotic response of the N' plants is coded in the coat protein gene.

OP5.8

THE FUNCTIONS OF THE 130K/180K PROTEINS OF TMV IN VIRAL RNA REPLICATION.
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Tobacco mosaic virus (TMV) encodes at least three non-structural proteins and two of them, 130K and 180K proteins (the latter is produced by suppression of termination of the former), are suggested to be involved in viral RNA replication. To clarify their functions, we constructed several cDNA clones carrying mutations at or near the termination codon of the 130K protein gene and then converted them into the corresponding viral RNA by means of the in vitro transcription system. At first, their infectivities were examined by inoculation on tobacco plants. Three (two frame-shift and one non-sense) mutants carrying the intact 130K and defective 180K protein gene were not infectious, indicating that the 180K protein is essential for viral multiplication in tobacco plants. On the other hand, R2 mutant which has a tyrosine codon in place of the termination codon of the 130K protein gene and is supposed to produce the intact 180K protein but no 130K protein was infectious. For further analysis, we introduced R2 RNA into tobacco protoplasts by electroporation. In comparison with wild-type, the synthetic rate of plus-strand RNAs was reduced while that of minus-strand RNA was similar. From these observations, we discuss the roles of the 130K and 180K proteins of TMV in viral RNA replication.

OP5.9

ANALYSIS OF TMV MUTANTS WITH DELETIONS IN THE 3' NON-CODING REGION.
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The 200 nucleotides long 3' non-coding region of tobacco mosaic virus (TMV)-RNA contains three consecutive pseudoknots and the tRNA-like structure. This highly-structured conformation is well-conserved among tobamoviral RNAs in spite of their sequence divergence. To elucidate the biological role of the 3' non-coding region of TMV-RNA and to search for the functional meaning of the structural feature, we have constructed TMV-RNA derivatives in which various lengths of deletions were introduced in the 5'-proximal portion of this region by using the in vitro transcription system. The essential portion of the 3' non-coding region was surveyed by local lesion assay of the deletion mutants on tobacco plants. When all three pseudoknots were removed, infectivity was abolished. To scrutinize the essential region for viral viability, a variety of deletion mutants are now being assayed further.

OP5.10

FUNCTION OF THE 30K PROTEIN OF TMV.
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Tobacco mosaic virus (TMV) codes for three non-structural proteins (130K, 180K and 30K proteins) and the coat protein. The 30K protein has been thought to be involved in cell-to-cell movement of virus without any conclusive evidence. Further, whether the 30K protein is necessary for replication is not known at all.

To elucidate the function of the 30K protein, we constructed several mutants with a modified 30K protein gene using an in vitro system to produce an infectious TMV RNA from a cloned cDNA copy and analysed their ability for cell-to-cell movement and replication using tobacco plants and protoplasts. A mutant having a single base substitution (causing an amino acid change) found in the 30K protein gene of Lsl, the most characterized strain defective in cell-to-cell movement at a higher temperature showed the same ts phenotype as Lsl. Deletion mutants lacking the 30K protein gene and both the 30K and coat protein genes were viable in protoplasts. These observations show that the 30K protein is involved in cell-to-cell movement and dispensable for replication.

OP5.11

IN VITRO TRANSLATION OF COWPEA MOTTLE VIRUS RNA.
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Cowpea Mottle Virus (CMeV) is a single component, isometric plant virus. It is a extreme virulence in cowpea, but has been reported only from Nigeria. CMeV has a genomic single-stranded RNA of (+) polarity (M.W. 1.4×10^6 daltons) with no polyadenylated sequence at the 3'end. It has one capsid protein of 42-45K daltons.

CMeV RNA extracted from a purified virus preparation was translated in vitro in a messenger-dependent reticulocyte lysate. Maximum incorporation of ^{35}S-methionine was obtained when 1mM magnesium and 30mM potassium were added. One major protein was produced. It co-migrated with CMeV capsid protein in PAGE and was immunoprecipitated by capsid protein antibody.

Three segments of double-stranded RNA were obtained from CMeV infected cowpea leaves. When the total dsRNA was denatured with DMSO and added to a reticulocyte lysate in vitro translation system, a 42-45K protein which immunoprecipitated with CMeV antiserum was obtained. The cistron for the translation product appears to be near the 5'terminus.

OP5.12

CITRUS PSOROSIS DISEASE AGENT: ASSAY AND PARTIAL PURIFICATION
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Known from long ago as a citrus disease in several countries, psorosis has steadily expanded in Argentina causing at present the loss of 5-8 % orange and grapefruit trees per year. Although the etiological agent has not been identified yet, psorosis is considered as a viral disease. As a first step towards a better agronomical control of the disease we started a project aimed at the purification and characterization of the putative "Psorosis Virus".

Psorosis was mechanically transmitted from citrus to Chenopodium quinoa, inducing local necrotic rings proportional -in number- to the relative concentration of the inoculated extract. Grafting transmission to several citrus followed by assay on Ch. quinoa showed that the most infective extracts were those obtained from Pineapple sweet orange and Eureka lemon. Symptomatic young leaves from these two citrus hosts were ground and homogenized in buffer. PEG precipitation and high speed centrifugation rendered infective pellets, although much of the infectivity was lost during the process. Electron-microscope observation of high speed pellets derived from infected and healthy leaves did not show consistent differences which may suggest that an elongated virus is not involved.

Several strategies are being tested for further purification.

OP5.13

ROLE OF GROWTH HORMONES IN BREAKING BUD DORMANCY OF VIRUS INFECTED STEM CUTTINGS OF EUPHORBIA PULCHERRIMA. C.L. Mandahar, S. Nath. Department of Botany, Panjab University, Chandigarh-160014, India.

Euphorbia pulcherrima grows vegetatively during summer from April to October and enters a state of dormancy in winter from October to March. During the dormant period, the bud on stem cuttings taken from healthy stocks remain dormant while those on stem cuttings from poinsettia mosaic virus infected plants grow vegetatively into leafy shoots since virus infection offsets the induction of bud dormancy. Quantitative and qualitative changes in growth regulators in healthy and infected stem cuttings at monthly intervals from October to March were studied. Offsetting of bud dormancy in virus infected stem cuttings was associated with markedly higher endogenous gibberrllins, particularly gibberellic acid, and cytokinins, particularly zeatin, content as well as very low abscisic acid endogenous content. Auxins did not play any role in this phenomenon. Exogenous application of the growth hormones confirmed these conclusions.

OP5.14

IDENTIFICATION OF SOURCES OF RESISTANCE TO TOMATO SPOTTED WILT VIRUS IN ARACHIS SPECIES.

E. Scholberg, A.S. Reddy, S.L. Dwivedi, D.V.R. Reddy and J.P. Moss. Legumes Program, International Crops Research Institute for the Semi-Arid Tropics, Patancheru 502 324, India.

Bud necrosis disease of peanut (A. hypogaea) caused by tomato spotted wilt virus (TSWV) has recently been reported to be economically important in several countries and is currently the most important virus disease of peanut in India. Several peanut germplasm lines, wild Arachis sp. and interspecific hybrid derivatives were screened for field resistance to TSWV. Genotypes with least disease incidence were evaluated for resistance to TSWV under laboratory conditions by mechanical inoculation. Virus concentration in inoculated plants was tested by infectivity assays and ELISA. All the ten peanut genotypes could be infected. The two interspecific stable tetraploid progenies 2159 and 2171 showed higher concentration of virus. Two breeding lines GBPRS 312 and GBPRS 138 had some degree of tolerance to TSWV. A. chacoense was resistant to the virus in mechanical sap inoculations. However, several crosses of A. chacoense and A. hypogaea, were found to be susceptible to TSWV. Efforts are underway to transfer resistant genes from A. chacoense to A. hypogaea.

OP5.15

CHARACTERIZATION OF VIRAL RNA POLYMERASE OF RICE BLACK-STREAKED DWARF AND RICE RAGGED STUNT VIRUSES. I. Uyeda, L. Sang Yong, H, Yoshimoto, E. Shikata. Department of Botany, Faculty of Agriculture, Hokkaido University, Sapporo, Japan.

Rice black-streaked dwarf (RBSDV) and rice ragged stunt (RRSV) viruses belong to plant reovirus subgroup 2 and 3, respectively. Although virion associated RNA polymerase was expected for viruses in Reoviridae, it has not been fully characterized nor detected in these subgroups. We detected RNA polymerase activity in the purified subviral particles of RBSDV and the virions of RRSV in vitro.

Temperature, pH, and $MgCl_2$ concentration critically affected the activity and these conditions were optimized for each virus. Synthesized RNAs hybridized with the denatured genomic RNAs migrated identically to corresponding genomic dsRNAs in polyacrylamide gel electrophoresis, showing these viruses transcribed full length copies of their genomic RNAs. S-adenosyl-L-methionine and chymotrypsin significantly stimulated the activity of RRSV.

OP5.16

TWO VIRUSES ISOLATED FROM PATCHOULI PLANTS. K.T.Natsuaki*, K.Tomaru**, C.S.Cheng**, and K.Nakata**. Lab. Tropical Plant Protection* and Nodai Research Inst.**, Tokyo University of Agriculture, Tokyo, Japan.

Patchouli (Pogostemon patchouli Pellet.) is tropical aromatic Labiatae plant and known to show mosaic symptoms in Indonesia and The Philippines. Two apparently undescribed viruses were detected from patchouli grown experimentally in Japan and designated patchouli mottle virus (PaMV) and patchouli mild mosaic virus (PaMMV) tentatively.

PaMV was sap transmissible and infected patchouli, Chenopodium amaranticolor, Tetragonia expansa and Sesamum indicum. PaMV had long flexuous particles ca. 700-800 nm and cytoplasmic inclusions. The virus was not related to watermelon mosaic virus, bean yellow mosaic virus and turnip mosaic virus serologically.

PaMMV was sap transmissible to 5 families including patchouli, Nicotiana glutinosa, Chenopodium amaranticolor, Gomphrena globosa and Tetragonia expansa. PaMMV particles were spherical ca. 28 nm in diameter and observed in the cytoplasm of mesophyll cells. PaMMV was not related to broad bean wilt virus, cucumber mosaic virus and some Nepoviruses occurred in Japan.

Virus free patchouli plants were obtained by meristem tip culture. Patchouli plants are propagated vegetatively, and the meristem tip culture is useful to produce healthy seedlings.

OP5.17

MOLECULAR CLONING OF RICE DWARF VIRUS GENE SEGMENT 9 AND 10. I. Uyeda, T. Matsumura, T. Sano, K. Ohshima, E. Shikata. Department of Botany, Faculty of Agriculture, Hokkaido University, Sapporo, Japan.

Rice dwarf virus (RDV) belongs to plant reovirus subgroup 1 and its genome consists of 12 segments of dsRNA. Gene segments 9 and 10 were cloned into PstI site of E. coli plasmid pBR 322 after homopolymeric tailing. cDNA inserts with apparent lengths of about 1300-1400 nucleotides were obtained for both segments 9 and 10. dscDNA of segment 10 was also made from viral transcript synthesized in vitro by a method of Gublar and Hoffman (1983). One clone of about 1,000 nucleotides in length was found to contain complete 5'end of the transcripts by primer extension and dideoxy sequencing analyses of both transcripts and the cDNA clone. Sequence analysis of the segment 10 will be presented.

OP5.18

COMPLETE GENE STRUCTURE OF MUNGBEAN YELLOW MOSAIC VIRUS DNA.

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Mungbean yellow mosaic virus ssDNA was converted into dsDNA state with reverse transcriptiase. Major two components DNA (A and B) and minor component DNA(C) were detected and physical maps for DNA A and B were made. By molecular cloning of MYMV dsDNA, full length DNA A or B was cloned into E.coli plasmid pBR322. Total nucleotide sequence of DNA A(2685ntds) and B(2723ntds) were determined by two methods of Maxam Gilbert's and M13 dideoxy's.

Conserved sequence regions(ca.200ntds) revealed in A and B. Unique hairpin structures, composed of GC rich stem and AT rich loop, are discovered in the conserved regions. DNA A and B have nine open reading frames (m.w. of potential protein >8,000). ORFs1-4 (m.w. of proteins >28,000) are coded in virion DNA A,B and their complementary sense strands. All of the ORFs1-4 have regulatory signals for RNA synthesis,respectively. Comparisons of nucleotide sequences and amino acid sequences of protein coded in each ORF are discussed among geminiviruses.

OP5.19

CYMBIDIUM MOSAIC VIRUS AND ODONTOGLOSSUM RINGSPOT VIRUS IN MALAYSIAN ORCHIDS. Norani Abdul-Samad. Department of Biochemistry and Microbiology, University of Agriculture Malaysia, 43400 UPM, Serdang, Selangor, Malaysia.

Cymbidium mosaic virus (CyMV) and Odontoglossum ringspot virus (ORSV) were the two major viruses infecting several orchid cultivars and hybrids in Malaysia which include Dendrobium sp., Cattlya sp., Oncidium sp., Mokara sp., Aranthera sp., Aerides sp., Aranda sp., Cymbidium sp and Phalaenopsis sp. The virus/viruses could singly or doubly infect the plants and infected plants were found in almost all orchid growing areas in Malaysia. The viruses were also present in orchid plants imported into the country. Identity of both viruses was confirmed by immune electron microscopy (antisera to CyMV and ORSV were kindly supplied by Professor F.W. Zettler, University of Florida, USA). CyMV and ORSV had a modal length of 490 nm and 300 nm respectively. CyMV produced local lesion on inoculated leaves of Cassia occidentalis and Chenopodium amaranticolor; while inoculated leaves of C. amaranticolor and Gomphrena globosa developed local lesion with ORSV. Disease symptoms on the infected leaves varied from severe necrosis to symptomless. The viral infection may affect the number, size and quality of flowers. The viruses were weakly immunogenic when injected into rabbits with titer in gel immunodiffusion test of 64 for ORSV and 128 for CyMV.

OP5.20

PURIFICATION AND IDENTIFICATION OF VIRAL GENOMES FROM HYBRID MUSHROOM AGARICUS BISPORUS. Y.H. Kim, R.F. Bozarth. Department of Life Sciences, Indiana State University, Terre Haute, Indiana 47809, USA.

Four different sizes of viral genomes from the cultivated hybrid-mushroom, Agaricus bisporus, virus has been extensively characterized and identified to be shown genomes as different sizes of double-stranded(ds)RNA and a unique double-stranded(ds)DNA segment. The purification procedures entailed extraction at three different pHs (6,7, and 8) of sodium phosphate buffer, follwed by PEG-NaCl precipitation, and sucrose density-gradient centrifugation.

SDS and phenol/chloroform phase extraction, agarose and SDS-polyacrylamide gel electrophoretic analyses revealed that this hybrid-mushroom virus(HMV) contain virus-like particle with three different sizes of dsRNA, a single DNase-sensitive segment and three different size of capsid polypeptides. This unique DNA segment was hydrolyzed by pancreatic DNase I, but not by pancreatic RNase A in high ionic strength buffer. Further, this distinct dsDNA segment was treated with several DNA- or RNA-sensitive enzymes. RNA-sensitive enzymes did not hydrolyze the DNA segment. This segment of genome was extracted from a unique peak of sucrose density-gradient centrifugation. This fractions of virus was investigated under the electron microscopy. Circumstantially, these results suggest that a distinct virus may have a novel mycovirus containing a dsDNA genome.

OP5.21

ALFALFA MOSAIC VIRUS - A NOVEL PATHOGEN IN AUSTRALIAN PASTURES. D. Dall, J. Randles, R. Francki. Department of Plant Pathology, University of Adelaide, Waite Agricultural Research Institute, Glen Osmond, South Australia, 5064.

Alfalfa mosaic virus (AMV) is increasing in both range and incidence in Australia, probably as a result of the accidental introduction of three important aphid vectors. Although common in the northern hemisphere, AMV is thus a novel pathogen to Australian pasture species, most of which have been selected on criteria other than tolerance to this virus.

Experiments with the important annual medic species *Medicago truncatula* suggest that AMV infection causes major reductions in both herbage production and root growth and nodulation. Field trials have shown that two *M. truncatula* cultivars - Jemalong and Sephi - suffered growth reductions of 40 to 80% when infected by AMV.

Screening of *M. truncatula* cultivars has revealed significant differences in relative susceptibilities to AMV infection, although no cultivar has been found to be completely resistant. Breeding of tolerant/resistant cultivars should now be viewed as a priority concern in Australian agricultural research.

OP5.22

STIMULATION OF GROWTH IN ALFALFA BY INFECTION WITH ALFALFA MOSAIC VIRUS. K. A. Miczyński, C. Hiruki. Department of Botany, Academy of Agriculture, Kraków, Poland and Department of Plant Science, University of Alberta, Edmonton, Alberta T6G 2P5, Canada.

Commercial alfalfa cultivars usually show considerable variation in their response to infection with alfalfa mosaic virus (AMV) due to their genetic complexity. The authors studied the effect of a severe strain of AMV (A-515) on forage yield and development of several alfalfa cultivars during repeated cutting and regrowth in the greenhouse and under controlled environment conditions. Although most AMV-infected plants showed significant growth reduction, there was often a small number of AMV-infected plants that were highly tolerant to infection under these stress conditions. In several cases, such plants gave higher dry-weight yields of forage than healthy controls, indicating a stimulatory effect of AMV infection on plant growth. The expression of the growth stimulation was both cultivar and temperature dependent. It was more pronounced in cv. Beaver than other cultivars and was promoted by elevated temperatures.

OP5.23

VIRUS DISEASES OF MAPLE TREES. G. Erdiller. Department of Plant Protection, Faculty of Agriculture, University of Ankara, Ankara, Turkey.

An infection agent which was first detected on maple trees located in Ankara, Keçiören-Kalaba province, had distributed recently. It has also been detected in parks and gardens of Afyor, Eskişehir, Denizli and İzmir. According to the results of the mechanical inoculation from infected trees to different host plants, serological tests and immunosorbent electron microscopy (ISEM), this causal agent which later caused great epidemics on maple trees was determined as a virus.

In mechanical inoculations, it has caused symptoms on Chenopodium amaranticolor, C.quinoa, C.murale, Nicotiana tabacum, N.glutinosa and Cucumis sativus.

It has also caused positive reactions with Cucumer Mosaic Virus (CMV), Sowbane Mosaic Virus (SMV), and Arabis Mosaic Virus (AMV) antisera in serological tests and ISEM.

In electron microscopy observations, isometric particles in 26-30 nm. diameter were detected.

OP5.24

THE BIOLOGICAL AND SEROLOGICAL STUDIES WITH TOBACCO MOSAIC VIRUS STRAINS ISOLATED FROM VARIOUS CULTIVATED PLANTS. U.Yorgancı,S.Erkan. Department of Plant Protection,Faculty of Agriculture,University of Ege,Izmir,Turkey 35100.

In the present study,11 isolates (4 from tomato,3 from tobacco,1 from chrysanthemum,1 from eggplant,1 from freesia and 1 from pepper) which had been detected to be the strain of tobacco mosaic virus in the previous investigations were comparatively examined from the standpoint of their reactions on several test and cultivated plants and their serological properties.

It was observed that these isolates having fairly different symptoms on cultivated plants caused similar symptoms on all test plants except for pepper. Moreover, it was determined that the serological properties of isolates in question were almost the same.

OP5.25

CUCUMBER MOSAIC VIRUS RNA 3 AS A FACTOR AFFECTING SYMPTOMS. Yau-Heiu Hsu, Chung-Chi Hu and Ren-Jong Chiu. Agricultural Biotechnology Laboratories, National Chung Hsing University, Taichung, Taiwan

Two cucumber mosaic virus (CMV) strains, NT9 inciting severe mosaic with high virus titer on Nicotiana tabacum L. cv. Van-Hicks and M48 causing symptomless infection and low virus titer on same host, were used to construct the pseudorecombinants for genetic analysis of symptom formation. Pseudorecombinant $N_1N_2M_3$ was constructed by mixing gel separated RNAs 1 and 2 from NT9 with RNA 3 from M48 and $M_1M_2N_3$ by mixing RNAs 1 and 2 from M48 with RNA 3 from NT9.[1,2] Each construction was inoculated onto Chenopodium quinoa, cloned by serial single-lesion passages through this host and propagated in tobacco. The true nature of the pseudorecombinants was checked by gel analysis of RNAs, peptide mapping of capsid proteins and back crosses to restore the parental virus strains. The symptoms on most of host species inoculated with $N_1N_2M_3$, $M_1M_2N_3$ and their parental strains gave evidence indicating a close association of CMV RNA 3 with symptoms. The symptom of NT9 was suppressed on tobacco by prior inoculation with either M48 or $N_1N_2M_3$. The virus concentration in tobacco was not affected by exotic RNA 3. Of the two parental CMV strains, RNA 5 was only detected in M48, but, the addition of RNA 5 to NT9 caused no reduction in symptom severity of the latter virus strain.

OP5.26

IMMUNODETECTION OF THE TRANSPORT FACTOR (30K) OF TOBACCO MOSAIC VIRUS IN INFECTED PLANTS.
T. Godefroy-Colburn, M.-J. Gagey, O. Moser, M. Ellwart-Tschürtz(•), H. Nitschko(•), C. Stussi-Garaud. Laboratoire de Virologie, IBMC du CNRS, Strasbourg, France and (•) Biological Institute, Dept of Botany, University of Stuttgart, Stuttgart, FRG.

The 30K non-structural protein of tobacco mosaic virus (TMV) was detected on Western blots, using an antiserum to a synthetic C-terminal nonapeptide. This protein was followed in inoculated leaves during systemic infection of Nicotiana tabacum (cv. Samsun). It was transiently found in a crude membrane fraction but accumulated more stably in cell walls. This location is consistent with the role of the 30K protein as "transport factor" (TF) which mediates cell-to-cell movement of TMV, and may be a general characteristic of plant viral TFs. Another example is the putative TF of alfalfa mosaic virus, P3 (1, 2). It is conceivable that in hypersensitive hosts, interaction of these proteins with (a) specific component(s) of the wall triggers the plant response to viral infection.

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(1) T. Godefroy-Colburn et al. (1986) J. Gen. Virol. 67, 2233-2239.
(2) C. Stussi-Garaud et al. (1987) J. Gen. Virol. in press.

OP5.27

INTERACTION OF BARLEY STRIPE MOSAIC VIRUS AND TOBACCO MOSAIC VIRUS IN BARLEY LEAVES. S. Ouchi, A. Muraoka, Y. Shimazu, S. Fukaya and Y. Takami. Plant Pathology, Faculty of Agriculture, Kinki University, Higashi-Osaka, Japan.

Multiplication of tobacco mosaic virus(TMV) and barley stripe mosaic virus(BSMV) in barley leaves was studied to elucidate interactions of these viruses as an approach to the molecular mechanism of viral replication in plants. TMV did not multiply in barley leaves, but multiplied to a significant quantity when co-inoculated with BSMV. Multiplication and translocation of both viruses were estimated by direct quantitative determination and indirect immunofluorescence assay. TMV multiplication in inoculated primary leaves was almost negligible comparing with that in leaves which had developed after co-inoculation, suggesting that BSMV helped TMV to translocate to other parts of the inoculated plants. Double inoculation experiments with protoplasts prepared from healthy barley mesophyll tissue indicated that TMV replicates in these protoplasts provided BSMV was present in the same cell. However, several other viruses were allowed to multiply neither in leaf tissues nor in protoplasts even if BSMV was co-inoculated. These results indicate that BSMV complements, by some unknown mechanism, the complex replication processes of certain specific virus(es). Complementation analysis with viral RNAs suggested that BSMV RNA-2 may codes information essential for the replication of TMV or for the repression of resistance mechanism of the host cells.

OP5.28

PARTICIPATION OF TMV 30K ORF IN LOCAL LESION FORMATION.
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Infection of N'-gene tobaccos with TMV causes either systemic infection or local lesion formation, depending on the virus strain used. By mutagenizing partially stripped virus particles (PSV) the efficiency to cause local lesion strains from systemic ones was studied as a function of stripping degrees. By this "stripping mapping" direct involvement of the 30k ORF in lesion formation was found. Artificial subgenomic mRNA still producing 30k in-vitro translation products but devoid of infectivity and capability to produce 126k and 183k polypeptides was prepared from RNase-treated PSV by phenol extraction. By complementation analysis such subgenomic mRNA of wild type TMV was found to reduce lesion size while that from local-lesion mutants did not. - The question whether active translation of the 30k ORF is required to suppress necrogenesis is still open. It has been studied by protein-chemical comparison of wild-type and local-lesion mutant's 30k in-vitro translation products. So far only two out of 37 "single-hit" HNO_2 mutants exhibited changes in 30k polypeptide structure. In some of the other cases "silent" mutations within the 30k ORF have been identified by nucleotide sequencing. While involvement of the 30k ORF (or some nucleotide sequences within it) in necrogenesis (possibly in its suppression) became clear other mechanisms for triggering necrogenesis in N'-gene hosts still cannot be excluded.

OP5.29

HOST-VIRAL GENOME INTERACTIONS OF CAULIFLOWER MOSAIC VIRUS. R. Stratford and S. Covey.
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Plant and viral genomes interact during infection to determine symptom expression. Isolates of Cauliflower Mosaic Virus, (CaMV) that produce different symptom types exist in nature. The molecular basis of symptom expression by two such isolates has been studied. Study of the overall symptom type and rate of rise of viral DNA has been complemented by analysis of changes in host gene expression at the level of both protein and mRNA populations.

CaMV isolate Cabb B-JI infection was characterized by severe chlorosis, stunting of the host plant and a rapid rise in viral DNA levels. Specific changes in host gene expression were detected. In contrast, CaMV isolate Bari 1 was characterized by the formation of dark green islands, rather than chlorosis with a much slower rise in viral DNA. Again, specific changes in host gene expression were found to occur. The contribution of the virus genome to symptom expression will be discussed.

OP5.30

INTERFERENCE BETWEEN COMOVIRUSES IN MIXED INFECTIONS OF COWPEAS. K. Eastwell, G. Kalmar. Department of Biological Sciences, Simon Fraser University, Burnaby, B.C., Canada.

Cowpea mosaic virus - SB (CPMV) and cowpea severe mosaic virus - DG (CPSMV) are serologically related members of the comovirus group. Cowpea lines are available that are highly susceptible to CPSMV, but do not support the replication of CPMV. In one such cowpea line, CPMV induces resistance to CPSMV when the viruses are introduced simultaneously. We are investigating the molecular basis of this phenomenon.

A partial cDNA library of virus sequence was prepared in M13, and individual clones were selected that did not hybridize with the genomic RNA of the alternate virus. Where possible, hybridization was confirmed by sequence analysis. The desired fragments were subcloned into a plasmid vector at a site flanked by the T7 and SP6 promoters. We then synthesized, in vitro, RNA probes specific for either the positive or negative sense virus RNA sequences by catalyzing the reaction with the appropriate polymerase. To quantitate viral capsid proteins synthesized during mixed infections, monoclonal antibodies were prepared; from the bank of hybridomas generated, we have selected, for use in ELISA, those that produce antibodies specific for the virus strain that was used to immunize the mice. Using these molecular tools, we have quantitated each step in the replication of two related viruses in a mixed infection. These data enable us to speculate on the mechanism(s) by which one virus can limit the replication of a second related virus.

OP5.31

cDNA CLONING OF HOST AND VIRAL mRNAs FROM CAULIFLOWER MOSAIC VIRUS-INFECTED BRASSICAS. K. Saunders and S.N. Covey, Department of Virus Research, John Innes Institute, Colney Lane, Norwich NR4 7UH, U.K.

CaMV is a well characterized DNA plant virus regarding its replication strategy and its expression of viral genes. Information concerning its interaction with its host is lacking. We are investigating this phenomenon by adopting a molecular cloning strategy.

Poly(A) enriched mRNAs isolated from mock-infected and CaMV-infected turnips were reverse transcribed into cDNAs and cloned into a plasmid vector to generate a cDNA library. cDNA clones of host origin, arising or diminishing as a consequence of the viral infection, were selected by performing a series of differential hybridisations. To accomplish this the cDNA libraries were screened using cDNA made to mRNA from mock-infected and CaMV-infected turnips and to CaMV DNA.

The properties of host cDNA clones identified by this procedure will be presented along with cDNA clones of viral origin isolated during this study.

OP5.32

ULTRASTRUCTURE AND AUTORADIOGRAPHY OF MAIZE TISSUES INFECTED WITH BYDV. M.A.Favali[+],R.Osler[++] and N.Loi[++].
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In previous ultrastructural study (1) on leaves of maize plants infected with Barley Yellow Dwarf Virus (BYDV),a luteovirus phloem-limited and only transmitted by aphids,we observed that some alterations,such as a substantial accumulation of starch in the chloroplasts of the bundle sheath cells, were common to all plants tested,while others were peculiar of each line.In an attempt to throw some light on these problems and in order to know where the virus particles were synthesized,we undertaken a combined morphologic and autoradiographic study on leaves and roots (never observed before) of several lines of maize either healthy or infected with BYDV. The samples were exposed to ^{3}H-uridine for various times and examined by electron microscopic autoradiography to detect the possible sites of virus synthesis and/or assembly.
Our results demonstrated that ^{3}H-uridine was incorporated almost exclusively into nuclei and cytoplasm of the phloem cells,where the virus particles and the tipical alterations were also observed.

(1)Favali M.A. et al., 1985.Mitteilungen aus der Biologischen Bundesanstalt:228:21-26.

OP5.33

EARLY EVENTS IN PROTOPLAST INFECTION BY TMV.
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Institute of Biology and Pedology, Far-Eastern Science Centre of the Academy of Science of the USSR, Vladivostok, USSR.

Uptake of TMV by isolated tobacco protoplast was studied using the scanning and transmission electron microscopy. The obtained data are consistent with the idea of the endocytic uptake of viral particles and their aggregates. Two steps of virus endocytosis (adsorption and proper uptake) were brought out by experiments with metabolic inhibitors and low temperature.
The ratio of ^{32}P : ^{14}C radioactivity in protoplasts was proved to be constant during the uptake of TMV preparation labelled by ^{32}P in nucleic and by ^{14}C in protein moiety. The first steps of TMV reproduction were accompanied by modifications in endoplasmic reticulum.

OP5.34

ELECTROPORATION-MEDIATED TRANSFER OF TOBACCO RINGSPOT AND SATELLITE TOBACCO RINGSPOT VIRUS RNA INTO PLANT CELL PROTOPLASTS. F.E. Christensen and G.A. Prody. Department of Chemistry, Western Washington University, Bellingham, U.S.A.

Tobacco ringspot virus (TobRV) is a nepovirus that consists of two genomic RNAs (RNA1 of about 7000 bases and RNA2 of about 3500 bases). During laboratory transfers, a third type of RNA, of small molecular weight, becomes encapsidated in the same protein coat, and reduces the severity of the symptoms normally associated with TobRV infected plants. This small RNA, termed satellite TobRV RNA (STobRV RNA) has a monomeric length of approximately 360 bases, and apparently does not arise from the genomic TobRV RNA. To further study this system, isolated TobRV and STobRV RNAs were transfered by electroporation into protoplasts of Nicotiana tabacum. Virus replication was evidenced by indirect immunofluorescence and by peroxidase activity linked to antibodies against TobRV coat protein. Satellite TobRV RNA replication was monitored using dot blot hybridization analysis and by local lesion assays on cowpeas (Vigna unguiculata Walp. California Black-eye 5).

OP5.35

TMV UPTAKE AND MULTIPLICATION IN PROTOPLASTS ISOLATED FROM LEAVES OF HYPERSENSITIVE TOBACCO PLANTS REVEALED THE ACQUIRED RESISTANCE.

V. I. Malinovsky, Yu. N. Zhuravlev.
Institute of Biology and Pedology, Far-Eastern Science Centre of the Academy of Sciences of the USSR, Vladivostok, USSR.

^{14}C-TMV uptake and multiplication of TMV in inoculated protoplasts isolated from the leaves with local (LAR) and systemic (SAR) acquired resistance of hypersensitive tobacco plants cv. Xanthi nc. were studied. Absorption of TMV by LAR protoplasts was found to be enchanced, SAR protoplasts absorbed TMV as much as protoplasts isolated from the leaves of healthy plants.
TMV reproduction in LAR and SAR protoplasts was suppressed. The level of suppression depends on the number of local lesions formed after inducing inoculation and on the accumulation of virus inhibitors in protoplasts.

OP5.36

A [illegible] FOR FLEXUOUS POTATO VIRUSES.
S.M.Paul Khurana, K.M. Singh, I.D. Garg, Shiv Kumar, Divn. of Pl.Pathology, Central Potato Research Institute,Simla, India.

The flexuous viruses get aggregated/fragmented during purification. A number of purification schedules were evaluated for flexuous potato viruses and a combined scheme developed. Nicotiana debneyii was found to be an ideal propagation host for PVY and PVB; tomato for PVY and tobacco cv. Havana-425 for PVY. Extraction of leaves, frozen for 1h, was done in 1:3 (W/V) of 0.2M Na_2HPO_4-0.02M citrate buffer (pH 8.5-9.0) having 0.1% $DIECA$+0.5mM EDTA and 0.5% 2-mercaptoethanol. The sap, squeezed through nylon/muslin cloth, was centrifuged (6,000 rpm, 10 min) and supernatant clarified with calcium phosphate in situ (by adding 0.2M Na_2HPO_4 1/20th+1.0M $CaCl_2$ 1/100th vols with 30 min stirring) followed by centrifugation (5000 rpm, 10 min). The supernatant obtained was adjusted to PEG-6000 4%+NaCl 0.8% (W/V) with 1h stirring. The pellet obtained, was suspended and reprecipitated using 4% PEG+1% NaCl, or 6% PEG alone for 2h. The virus was repelleted and resuspended in buffer (1ml/5g of tissue), kept overnight and clarified by 2,000-2,500 rpm, 10 min centrifugation. The virus was finally pelleted by ultracentrifugation (1,05,000g, 1.5h). The differential centrifugation was repeated. Under electron microscope the virus preparations were found apparently free from contaminants, aggregation or fragmentation of virions irrespective of any of potato viruses X,S, M Y purified by this method yielding good quantities.

OP5.37

INACTIVATION OF CAULIFLOWER MOSAIC VIRUS BY A PHOTOACTIVATABLE COTTON PHYTOALEXIN. Tzeli J. Sun, Ulrich Melcher and Margaret Essenberg, Department of Biochemistry, Oklahoma State University, Stillwater OK 74078-0454 USA.

Upon irradiation, 2,7-dihydroxycadalene (DHC), a phytoalexin produced by cotton in response to bacterial infection, inactivated virions of cauliflower mosaic virus (CaMV). The treatment destroyed infectivity observed either as local lesions on inoculated turnip leaves or as systemic infection of turnip plants. Neither irradiation alone nor DHC alone destroyed infectivity. Irradiation of a suspension of CaMV virions in DHC greatly decreased the electrophoretic mobility of 39 and 43 kilodalton coat protein fragments, but not of a 37 kilodalton fragment. The treatment also resulted in a reduction of the extractability of the DNA. Irradiation of a DHC-CaMV DNA solution resulted in numerous nicks in the CaMV DNA. The nicked DNA was, however, as infectious as the untreated DNA. We suggest that the inactivation of CaMV virions by DHC is due to the cross-linking of the nucleic acid binding domain of the coat protein to the viral DNA. (Supported in part by the Oklahoma Agricultural Experiment Station, the Herman Frasch Foundation and the National Science Foundation Grant PCM 83-16759.)

OP6.1

INFECTIOUS PANCREATIC NECROSIS CARRIER INCIDENCE IN ARCTIC CHAR POPULATIONS AND EXPERIMENTAL PATHOGENESIS. T. Yamamoto, Department of Microbiology, University of Alberta, Edmonton, Canada and B. Suter, Freshwater Institute, Fisheries and Oceans, Winnipeg, Canada.

Arctic char inhabiting the western tributaries of the Mackenzie River in the Northwest Territories, Canada are carriers of IPN virus. An examination of the char population over a 5-year period has shown the virus to be consistently detectable in over 40% of the population. The virus is also present in char populations in streams draining the northern regions of the Yukon and in the Beaufort Sea at Shingle Point.

Studies by immersion infection of fingerling salmonids have shown that the virus is pathogenic to varying degrees depending on the species and age of fish. Timed infection analysis revealed that an important mode of entry and replication of the virus site is the gill tissue. The virus was subsequently present in the pyloric serosal layers, followed by spread into the pancreatic and kidney tissues. Particles in membrane bound vesicles have been observed in the intestinal mucosal cells but replication in those cells is limited.

Infection experiments show that there is a marked variation in the replication, extent of virus spread, and pathogenesis in different individuals within the same population with some fish being completely refractory to infection, others exhibiting high titers of virus in many organs 3 days following infection. Mortalities are usually associated with high titers of virus present in many organs. Surviving fish may have no detectable virus or variable levels of virus.

OP6.2

STUDIES OF POLYNUCLEOTIDE-INDUCED INTERFERON EFFECTS IN SEVERAL POIKILOTHERMIC TISSUE CULTURE/VIRUS SYSTEMS. B. Middlebrooks, F. Tang, S. Huang. Department of Biological Sciences, University of Southern Mississippi, Hattiesburg, Mississippi, U.S.A.

Several poikilothermic tissue culture/virus systems, including (1) channel catfish ovary cell line (CCO)/channel catfish virus (CCV), brown bullhead cell line (BB)/CCV, (3) bluegill fry cell line (BF-2)/vesicular stomatitis virus (VSV), (4) BB/VSV, (5) fathead minnow cell line (FHM)/golden shiner virus (GSV), and (6) FHM/tadpole edema virus (TEV), were used to study virus inhibitory effects of interferon(s) induced by various polynucleotides (primarily poly I:C). These effects were studied in cultures producing endogenous interferon in response to polynucleotide exposure and in cultures treated with exogenous, homologous or heterologous, interferon (produced in other cultures in response to polynucleotide exposure). The minimum time of exposure to polynucleotide for induction of maximum levels of interferon production was determined to be 15 minutes. Optimum age of cultures for production of interferon was 4 days for most of the systems investigated. Virus inhibitory effect varied from a 0.5 $\log_{10}$ reduction in virus production in the CCV/VSV system producing endogenous interferon to a 3.0 $\log_{10}$ reduction in the BB/CCV system where cultures were first treated with exogenous, homologous interferon, then treated with poly I:C before exposure to virus.

OP6.3

SEROLOGICAL SPECIFICITY OF SALMONID ANTIBODY (IgM) TO INFECTIOUS PANCREATIC NECROSIS VIRUS. R.K. Kelly, O. Nielsen. Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, Canada R3T 2N6

Rainbow trout (Salmo gairdneri) express a 6 S natural serum inhibitor that neutralizes North American isolates of infectious pancreatic necrosis virus (IPNV) exhibiting a significant proportion (>2%) of cellular host range variants capable of plaque formation on the FHM cyprinid cell line. Serum levels of 6 S inhibitor and specific 16 S antibody (IgM) were followed over the course of immunization (i.p. with incomplete Freund's adjuvant) of mature trout with formalin-fixed, semi-purified IPNV (Buhl strain) or Buhl FHM-IPNV, a host range variant. Some trout produced little detectable antibody; whereas, others responded with maximum serum antibody titers (80% plaque neutralization) of >1:16000 to the Buhl FHM-IPNV. Mean serum titers in trout immunized with non-variant IPNV Buhl were lower and confirmed values reported by Sano et al. (Develop. Biol. Standard. 49: 63-70, 1981). Although antibody specificity was definitely not identical for individual trout, IgM induced to formalin-inactivated Buhl FHM-IPNV generally exhibited significantly cross-reactivity both to the wild type virus and to other IPNV (e.g. West Buxton, VR 299, Sp). The application of salmonid IgM is suggested for cross-neutralization testing of IPNV isolates.

OP6.4

FINE STRUCTURE MAPPING OF THE INFECTIOUS PANCREATIC NECROSIS VIRUS (IPNV) GENOME SEGMENT A CODING REGIONS. R. Duncan and P. Dobos. Department of Microbiology; University of Guelph, Guelph, Canada.

Full-length and truncated IPNV segment A cDNA was used to produce mRNA in vitro using T7 polymerase and the RNA was translated in rabbit reticulocyte lysates. Viral polypeptides were isolated by SDS-PAGE and electroelution and were characterized by peptide mapping using N-chlorosuccinimide. VP3 was mapped to the 3'-end of the RNA and is produced in vitro by internal translation initiation at methionine 16 or 17 (2307 or 2328 bases of the polyprotein ORF). Data obtained suggested that NS is a viral-coded protease and is produced in vitro by internal translation initiation at methionine 11 (1629 bases of the polyprotein ORF). The carboxy-terminus of NS is produced by cleavage of a precursor in the region encoded between bases 2289-2300. pVP2 is produced by translation initiation at methionine 1 (120 bases), the same initiation codon used to produce the polyprotein. The carboxy-terminus of pVP2 is generated by cleavage of the polyprotein in the region encoded between bases 1550-1650. NS is responsible for the cleavage generating the carboxy-terminus of pVP2. If NS is also responsible for the cleavage which generates its own carboxy-terminus, then Tyr-Leu dipeptides encoded by positions 2294 and 1592 (for the NS and pVP2 carboxy-termini respectively) represent the most probable sites for proteolytic cleavage.

OP6.5

FISH VIRUSES: ISOLATION OF A PICORNA-LIKE VIRUS FROM SMELT (Osmerus mordax). M. F. Li, A. R. Moore, M. McMenemy, Biological Sciences Branch, Department of Fisheries and Oceans, Halifax, Nova Scotia, Canada.

A new virus, presumptively identified as a Picornavirus has been isolated from landlocked smelt (Osmerus mordax) mortalities which occurred in a lake in New Brunswick. This virus was originally isolated in chinook salmon embryo (CHSE-214) cells inoculated with diluted tissue homogenates of pooled pyloric-ceca, spleen, and kidney and was subsequently found to produce cytopathic effect (CPE) in alewife kidney (AK), sturgeon heart (SH), bluegill fry (BF-2), and grunt fin (GF) cell lines. Electron microscopy revealed a small cytoplasmic icosahedral virus, 18-30 nm, and infectivity was not retained after ultrafiltration through a 25 nm cellulose ester filter. The virus is ether and pH 3 resistant, unstable at 56°C, replicates at 4, 15 and 18°C, and produces a unique CPE. Infectivity is not reduced by attempts to neutralize with a seven strain polyvalent infectious pancreatic necrosis virus (IPNV) antiserum. Replication is not inhibited by 10^{-4}M flurodeoxyuridine and acridine orange staining revealed typical RNA inclusions. The virus was not lethal for brook trout (Salvelinus fontinalis) but could be reisolated from tissue homogenates of pyloric-ceca, spleen and kidney 6 weeks after interperitoneal injection. Presumptive identification indicates the Enterovirus group and would be the first report of a virus from this group in fish.

OP7.1

A FOLLOW-UP STUDY OF HEPATITIS B VIRUS CARRIERS.
I. Tamura, H. Ichimura, Y. Itoh, O. Kurimura, T. Kurimura*. Institute of Clinical Research, Kure National Hospital, Kure, and *Department of Virology, Tottori University School of Medicine, Yonago, Japan.

To clarify the relation between hepatitis B virus (HBV) and hepatocellular carcinoma (HCC), a follow-up study of 1,614 HBV carriers (910 males and 704 females) was conducted at Kure National Hospital from 1970 to 1984. During the follow-up period (40.3 months on average), 247 HBV carriers died. Deaths from HCC and liver cirrhosis (LC) numbered 99 of 168 males (58.9%) and 38 of 79 females (48.2%), the chi-square test revealing no sex difference. Of 142 deaths from malignant neoplasm, HCC accounted for 52 of 96 males (54.2%) and 19 of 46 females (41.3%), the chi-square test revealing no sex difference. The adjusted odds ratio of death from HCC among HBV carriers (1,614) with respect to HBV non-carriers (176,909) in this hospital during the study period was 9.52 (males 7.94, females 13.39). The expected number of deaths was calculated based on the population of Hiroshima Prefecture (1978-1980) as a standard. The adjusted O/E (observed number/expected number) ratio of death from HCC was 6.66 for males and 12.13 for females, and the adjusted O/E ratio of death from LC was 5.41 for males and 11.02 for females. These findings suggest that HBV is a high risk factor of HCC, and unlike the general population, female carriers may have a rather higher risk of death from HCC and LC than male carriers.

OP7.2

THE CONTROL OF HEPATITIS B VIRUS INFECTION WITH VACCINE JAPANESE NURSERY SCHOOLS. J. Hayashi , S. Kashiwagi , H. Nomura , W. Kajiyama , H. Ikematsu , M. Noguchi[1], T. Shingu[2], K, Hayashida[3], M. Kaji , M. Kaji Jr[4]. 1) First Dep. of Internal Medicine, Kyushu University, 2) Dep. of Pediatrics, Nichinan Pref. Hospital, 3) Ohji Central Clinic, Ohji Co., 4) First Dep. of Internal Medicine Kurume University, Japan.

The efficacy of an inactivated hepatitis B vaccine was assessed among children in nursery schools where there was at least one hepatitis B surface antigen positive child with hepatitis B e antigen. Of the 496 children who completed the protocol, 243 (age 2.7 ± 1.1) in six of the nursery schools received three injections of the vaccine. In five other nursery schools, 253 (age 2.3 ± 1.0) did not receive the vaccine and were used as the control group. No side effects resulted from vaccination. Two doses of the vaccine induced antibodies in 70.8 % of the vaccinated children. A booster dose six months after the first dose induced antibodies in 82.3% of the recipients and markedly increased the proportion of recipients who produced high antibody titers. Although nine (4.4%) of the children in the control group were infected with hepatitis B virus and two of them became carriers, none of the vaccine recipients were infected. The vaccine appears to be safe, immunogenic, and efficacious in preventing infection with hepatitis B virus in nursery schools.

OP7.3

HEPATITIS B VIRUS SPECIFIC RNA TRANSCRIPTS IN THE HUMAN LIVER. H. Ikematsu , S. Kashiwagi , J. Hayashi , H. Nomura , W. Kajiyama*, M. Noguchi[1], T.Shingu[2], K. Hayashida[3], M. Kaji, M. Kaji Jr*. 1)First Department of Internal Medicine, Kyushu University, Fukuoka, 2) Dep. of Pediatrics, Nichinan Pref. Hospital, 3)Ohji Central Clinic, Ohji Co., 4)First Department of Internal Medicine, Kurume University, Kurume, Japan.

To investigate the gene expression of hepatitis B virus (HBV) in the human liver, we studied HBV specific RNA transcripts in human liver specimens. The liver specimens were obtained from patients who were operated to resect hepatocellular carcinoma. Total RNA was extracted from the non-tumorous parts of the resected livers. A total of 16 specimens were available, nine were positive for hepatitis B surface antigen (HBsAg) and seven were negative.

HBV specific RNA transcripts were found in the HBsAg positive patients, but not in HBsAg negative patients. HBV specific RNA transcripts included two major RNAs, approximately 3.6kb and 2.1kb in size. In some of the HBsAg positive patients, 3.6kb RNA was considered not to be a major transcript. The expression level of HBV specific RNAs showed differences among the patients, when compared using beta-actin messenger RNA as a marker.

These results suggest that there may be two major HBV specific RNA transcripts and the expression of HBV gene differs in each HBsAg positive patient.

OP7.4

IMMUNOCHEMICAL AND MORPHOLOGICAL PROPERTIES OF HBsAG, HBcAG AND HBeAG PRODUCED BY THE HUMAN HEPATOMA CELL LINE TRANSFECTED WITH THE CLONED HEPATITIS B VIRUS GENOME. H. Ohori[1], T. Akiba[1], K. Matsuda[1], T. Tsurimoto[2] and K. Matsubara[2]. [1]Department of Bacteriology, Tohoku University School of Medicine, Sendai, Japan, [2]Institute for Molecular and Cellular Biology, Osaka University, Osaka, Japan.

Immunochemical and morphological characteristics of hepatitis B virus (HBV) related antigens produced by the human hepatoma cell line which had been transfected with a recombinant HBV DNA were examined. Under the electron microscope, uniform particles with mean diameter of 22 nm for HBsAg and 27 nm for HBcAg were found in the culture fluid. The amounts of HBsAg, HBcAg and HBeAg in 300 µl of the cell lysate which was prepared by suspending 1.3×10^7 cells in lysis buffer and in culture fluid were determined semiquantitatively by the RPHA method. The titers of HBsAg, HBcAg and HBeAg were 2^{10}, 2^3 and 2^7 in cell lysate and 2^5, 2^4 and 2^6 in the culture fluid, respectively. The protein compositions of HBsAg and HBcAg were analyzed by immunoblotting with HBsAg- and HBcAg-specific antibodies. The HBsAg contained P24/GP27, P33/GP36 and P39/GP42 proteins and HBcAg contained only P21 protein. The proteins carrying HBeAg in cell lysate were P21 and P18, however, that in culture fluid was only P18. These results suggest that this established cell line can be used as a model system for analysis of gene expression of HBV in human hepatocytes.

OP7.5

EPIDEMIOLOGICAL STUDIES OF NON-A, NON-B VIRAL HEPATITIS AMONG OPERATED PATIENTS WITH AND WITHOUT BLOOD TRANSFUSION IN GENERAL SURGICAL UNIT. T. Kamiya[1], S. Kikuchi[2], K. Kikuchi[2] and H. Ohori[1]. [1]Department of Bacteriology, Tohoku University School of Medicine, Sendai, Japan, [2]Surgical Clinic of National Sendai Hospital, Sendai, Japan.

In order to estimate the incidence of non-A, non-B (NANB) hepatitis caused by blood transfusion, patients who received general surgical operation during 1971 to 1985 at National Sendai Hospital were divided into two groups; one was blood-transfused and the other was not. A total 4382 patients who were hospitalized for more than 3 months and whose sera were collected more than 4 times were included in this study.

Of these, 2732 patients received 1 to 30 units of blood and 264 patients (10.4%) developed NANB hepatitis (PTH). The rest 1650 patients who did not receive blood transfusion, 136 patients (8.2%) developed NANB hepatitis (NTH). It was noted that the transfusion volume did not affected the incidence of PTH. These results suggest that there may exist NTH with relatively high prevalence ratio among patients who were diagnosed to be PTH.

OP7.6

HUMAN HEPATITIS A VIRUS RECOVERY FROM SEEDED DRINKING-WATER. Divizia M; De Filippis P.; Di Napoli A.; Gabrieli R.; Panà A.
Department of Public Health - 2nd University of Rome - Tor Vergata - Via O. Raimondo 00173-Rome; Italy.

The recovery of seeded human hepatitis A virus in declorinated tap water was investigated. All the experiments were performed using flat membranes (47 mm diameter, 0.45um pore size) from different manufacturers and an ultrafiltration system by Millipore.

Each assay was performed using one liter of declorinated tap water, seeded with an unique dilution of virus.

The results clearly show different recoveries depending on the membranes.

The best recovery was obtained using Millipore (type HAWP) and electropositive Virosorb membranes (80-90%). Differently with Gelman membrane (type GN/6) the recovery was quite low (0.1%).

For the ultrafiltration assay we used the Minitan system manufactured by Millipore with a polysulfone membrane with a nominal molecular weight limit of 10,000 and three different operating conditions: 2 membranes (120cm^2), 4 membranes (240 cm^2) at two different income pressure. The results were practically the same with total recovery of the input virus.

OP7.7

IDENTIFICATION OF THE VIRUS OF HEPATITIS A IN WATERS WITH AN IMMUNOENZYMATIC METHODIC.

N.Giachino, L.Costa, V.Livoti, C.Maiorana, V.Onofaro, E. Pipito'.
Centro di Virologia Osp. R.MARGHERITA -MESSINA ITALY.

By following up the studies that allowed us in the past to identify the virus of the hepatitis A (HAV) in mussels ,with the EIA method, we have found a methodic that allows to individualize the virus HAV in waters with the same EIA method.The protocol of the study provides: - A experimental infection of tens of litres of water with a Know quantity of the virus HAV so as to obtain such a dilution as to render the virus non dosable.
- Concentration, by means of a system of an ultrafiltration with a tangential flow of the infected water up to a quantity of a few ml..
-Identification and titletion of the virus recovered with the immunoenzymatic method.
In the study, this protocol will be described in detail.
The pourpose of our study was that of finding a methodic that would permit to insert the research of the virus HAV in the protocols of studies for the diagnosis of drinkable waters.

OP7.8

An Epidemiological Wave of HBV in Lattakia, Syria ..... M. Morie , George Beylouni

Department of Microbiology, Tishrin University. Lattakia, Syria

Since no comprehensive study of HBV had been undertaken in Lattakia, the problem was investigated in selected risk group .

654 serum samples collected from individuals show monthly incidence of HBV infection .

Prevelence of HBV antibodies varied signifacantly between individuals from the low and high socio-economic classes of the society .

Our results indicate that epidemiological wave of HBV has dominated during 1986 in Lattakia .

OP8.1

CHARACTERIZATION OF MONOCLONAL ANTIBODIES AGAINST AKABANE SIMBU SEROGROUP BUNYAVIRUS. H. Akashi, Y. Inaba, H. Nagano, N. Kaneko, T. Tokui, Y. Tanaka. National Institute of Animal Health, Ibaraki, Japan.

Monoclonal antibodies(MoAbs) against Akabane virus were established by fuzing P3X63Ag8U1 mouse myeloma cells and spleen cells from BALB/c mice immunized with Akabane OBE-1 strain. Of the resulting 16 clones, 13 clones produced immunoglobulins(Igs) which precipitated the 100K glycoprotein (G_1) and 3 clones produced Igs which precipitated nucleoprotein(N). All MoAbs were characterized in enzyme-linked immunosorbent assay(ELISA), plaque reduction neutralization (NT) and hemagglutination inhibition(HI) tests against 4 Simbu serogroup viruses(Aino, Douglas, Peaton, Tinaroo) and 12 Akabane isolates.

Anti-N MoAbs reacted only with Tinaroo virus, but not with the other Simbu serogroup viruses. All anti-G_1 MoAbs were specific to Akabane virus, and 12 of 13 MoAbs had both NT and HI activities against the homologus OBE-1 strain. By competitive binding assay, at least 2 epitopes for N and 5 for G_1 were defined. Anti-G_1 MoAbs which reacted to the same epitope had unique reactivity while all anti-N MoAbs reacted almost same degree to all Akabane isolates. Most interesting result is that 3 isolates collected in the same place within 3 weeks had different reactive patterns detected by ELISA, NT and HI tests, showing great antigenic variation of Akabane virus in the field.

OP8.2

SELECTION BY VIRUS NEUTRALIZING MONOCLONAL ANTIBODY OF VARIANTS OF INFECTIOUS BURSAL DISEASE VIRUS AS A MEANS OF LOCALIZING ANTIGENIC REGIONS. M. Brown[1], A. Azad[1], J. Crooks[2], P. McWaters[2] and K. Fahey[2]. [1]CSIRO, Division of Protein Chemistry, Melbourne, Australia. [2]CSIRO, Division of Animal Health, Melbourne, Australia.

Infectious Bursal Disease Virus (IBDV) is a major pathogen affecting the worldwide commercial poultry industry, causing severe immunodeficiency in young chickens. The entire genome of IBDV has been cloned and sequenced, it consists of two segments of ds-RNA encoding 4 polypeptides of MW 52, 28, 32 and 90 kDalton.

In order to identify the number and exact location of epitopes involved in virus neutralization we have used a tissue-culture adapted IBDV to select virus variants resistant to a neutralizing monoclonal antibody (VN-MAb), designated 17-82.

We have used these variants in cross-neutralization experiments with other anti-IBDV VN-MAb's, to analyse the likely size and number of epitopes and analyse epitopes on an amino acid level by comparing viral cDNA sequences.

Since the MAb 17-82 is also host protective when passively transferred to chicks, we hope that the identification of its epitope will contribute towards vaccine development using heterologously expressed segments of genes encoding this region, or by a synthetic peptide approach.

OP8.3

ANTIGEN STRUCTURE ANALYSIS OF Ad35 HEXON BY MEANS OF MONOCLONAL ANTIBODIES. É.Adám, I. Nász, Cs.Kerékgyártó, A. Lengyel, J. Erdei, Gy.Berencsi, J.Fachet. Institute of Microbiology, Semmelweis Univ. Medical School, Budapest and Institute of Pathophysiology, Debrecen, Hungary.

A panel of monoclonal antibodies directed against Ad35 hexon was used for the analysis of the antigenic structure of the protein. Based on the data of indirect ELISA, passive hemagglutination, and gel diffusion experiments, different epitopes (epitope clusters) could be detected on the surface of Ad35 hexon. For the determination of distinct or partially ovelapping antigenic sites the ELISA additivity test was used. According to the reactivity patterns of the monoclonals studied with 12 different human adenovirus hexon types and with 2 ones of animal adenoviruses, the relationship is close between adenovirus types of the oncogenic subgenera (A and B), the other hexon types belonging to subgenera D, E, and C as well as types of animal origin showed looser relationship to the hexon of Ad35 hexon.

OP8.4

NEUTRALIZATION EFFICIENCY OF ANTI-POLIOVIRUS TYPE 1 ANTIBODIES DEPENDS ON THEIR BINDING SITES. A.G. Mosser and R.R. Rueckert. Biophysics Laboratory, University of Wisconsin, Madison, Wisconsin, USA.

We have studied twelve neutralizing monoclonal antibodies, previously mapped to three different binding sites by sequence analysis of escape mutants. Spanning distances between identical binding sites in adjacent pentamers are 50, 60 and 90Å for sites 2, 3C and 3B respectively. Titration curves for each of these antibodies against a standard preparation of virus have defined several types of neutralization profiles, which broadly divide the antibodies into efficient (less than 10^{-4} survivors in excess antibody) and inefficient (more than 10^{-3} survivors in excess antibody) neutralizers. Efficient neutralizers were only observed in site 3B, with a 90Å spanning distance. This may be because the spanning distances of the other two sites are too short to allow pentamer linking by the two arms of an antibody. The data so far fit the idea that pentamer linking is needed for efficient neutralization.

OP8.5

TESTING THE EPITOPE SPECIFICITY OF MONOCLONAL ANTIBODIES AGAINST NEWCASTLE DISEASE VIRUS BY COMPETITIVE FLUOROGENIC ENZYME IMMUNOASSAY. J.P. Wong, Y.M. Siddiqui, R.E. Fulton. Defence Research Establishment Suffield, Ralston, Alberta, Canada.

A test to determine epitope specificity of monoclonal antibodies (MCA) was developed for hybridoma clones producing antibodies against Newcastle Disease Virus. The virus was immobilized on nitrocellulose membranes of Millititer IM plates. Dilutions of MCA were added separately, as well as simultaneously, and bound antibody quantitated with alkaline phosphatase-labelled detector antibody and a fluorogenic substrate, 4-methylumbelliferyl phosphate. Additivity of fluorescence count, suggestive of participation of distinct viral epitopes, was measured fluorimetrically.

When additivity index was plotted against dilution of paired MCA, antibodies that recognized identical epitopes displayed non-additivity at high dilutions, followed by partial additivity and by total additivity at low dilutions. In contrast, MCA that recognized distinct epitopes exhibited total additivity throughout the curve.

This test is simple to perform and does not require estimation of concentration of MCA to saturate the antigen.

OP8.6

STRUCTURAL STUDY OF ADENOVIRUS TYPE 2 : ENZYMATIC CLEAVAGE AND USE OF ANTI-FIBRE AND ANTIPEPTIDE SERA

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Unité de Virologie Moléculaire, INSERM U. 233, Lille, France and European Molecular Biology Laboratory, Grenoble, France.

Peptides corresponding to the N- and C- extremities of the adenovirus 2 fibre polypeptide were synthesized, coupled on tetanus toxoid, and injected into rabbits. Two sera were obtained : the anti-N_{TT} serum and the anti-C_{TT} serum. The antipeptide sera were used to determine the site of anchorage of the fibre in the penton base : at low serum concentration, only the anti-C_{TT} serum revealed the complete penton (fibre linked to penton base) suggesting an assembly by the N-extremity of the fibre and the penton base. This was confirmed by a comparative analysis of in vitro interaction of cleaved and intact fibre with the penton base and immunological studies using antipeptide sera which suggested that the N-terminus of the fibre is involved in the contact between the two molecules. The cleaved fibre was the result of the limited proteolysis of the fibre which occurs spontaneously during the crystallization process. Sequencing of the protein extremities was performed and specific inhibitors led to the characterization of the cleavage site. The sera also allowed to identify fragments generated by hydrochloric acid cleavage of the fibre and to show i) specificity of the sera ii) a highly resistant antigenical conformation corresponding to a 48 K fragment which constitutes the 2/3 N-terminal part of the molecule. This is consistent with a shaft structure for this part of the fibre.

OP8.7

EFFECT OF RUBELLA VIRUS ON IMMUNE CELL FUNCTION. M. Barth and J.K. Chantler, Department of Medical Microbiology, Faculty of Medicine, University of British Columbia, Vancouver, Canada.

The ability of rubella virus (RV) to replicate in different human lymphoid cell types was investigated. CD4+(T4+)T-cells, CD8+(T8+)T-cells and B-cells, which had been stimulated by appropriate mitogens, were found to be permissive for RV replication, and yield high titres of progeny virus. In addition, viral antigen was shown to be expressed on the surfaces of CD4+/T4+ T-cells and CD8+/T8+ T-cells when a mixed population of peripheral blood mononuclear cells (PBMC) were infected and analysed by flow cytometry.

The effect of various RV strains on the ability of an Epstein-Barr virus (EBV)-transformed human B-cell line (CESS cells) to secrete IgG has been characterised. The HPV 77 strain of RV caused elevated secretion of IgG by acutely infected CESS cells as compared to uninfected CESS cells in the presence of B-cell differentiation factors (BCDF) produced by pokeweed mitogen (PWM) stimulated PBMC. In contrast, CESS cells, persistently infected by the M33 strain of RV, showed far less IgG production than uninfected CESS cells in the presence of lymphokine from PWM-stimulated PBMC. The effects of RV strains on the number of IgG secreting CESS cells has also been examined by an ELISA spot assay.

OP8.8

CELLULAR IMMUNE RESPONSES AGAINST SYNTHETIC PEPTIDES FROM THE LATENT MEMBRANE ANTIGEN (BNLF1-MA) OF EPSTEIN-BARR VIRUS. W. Jilg, S. Modrow, H. Mairhofer, H. Wolf. Max von Pettenkofer Institute, University of Munich, Munich, FRG.

B-lymphocytes immortalized by Epstein-Barr virus (EBV) are very efficiently controlled by the immune system. The target structure on the surface of EBV-infected B-lymphocytes recognized by cytotoxic T-cells is known as LYDMA (lymphocyte detected membrane antigen) which, at present, is characterized only functionally. The EBV-encoded latent membrane antigen (BNLF1-MA), which is expressed in all EBV-transformed lymphoblastoid cell lines, is a possible candidate for LYDMA. We have studied cellular immune responses in EBV-positive and negative persons against two synthetic peptides from this protein. The two peptides represent the amino acid sequences 42-53, which is expressed at the cell surface, and 69-81 situated in the interior of the cell. EBV-positive persons showed clear reactions in lymphocyte proliferation assays against the peptide from the cell surface but did not react against the inner sequence. Thus the BNLF1-MA sequence 42-53 seems to be an important structure for the recognition of EBV-infected lymphocytes by specific T-cells.

OP8.9

USE OF THE MONOCLONAL ANTI-REOVIRUS RECEPTOR ANTIBODY TO PREDICT THE OLIGOPEPTIDE NEUTRALIZING EPITOPE ON THE REOVIRUS TYPE 3 HEMAGGLUTININ. D. H. Rubin, F. Robey, W. V. Williams, and M.I. Greene: Veterans Administration Medical Center, and the University of Pennsylvania, School of Medicine, Departments of Pathology and Microbiology, Philadelphia, PA 19104 and the FDA 8800 Rockville Pike Bethesda, MD 20892.

We have previously shown amino acid homology between the type 3 reovirus hemagglutinin and a monoclonal anti-receptor antibody developed as an anti-idiotype against a neutralizing anti-hemagglutinin monoclonal antibody (Proc. Natl. Acad. Sci. USA 83:6578). We now have utilized synthetic peptides corresponding to the areas of sequence homology to immunize adult Balb/c mice. Groups of mice were immunized with either VL peptide, a 17 mer identical in amino acid composition to the anti-receptor antibody light chain second complementarity determining region (CDC II); VH peptide, a 13 mer comprising the CDR II of the heavy chain; and reo peptide, a 16 mer which corresponds to amino acids 317-332 of the type 3 virus hemagglutinin. As a control, an irrelevant 21 mer with no homology to either reovirus hemagglutinin or anti-receptor antibody was used. We found that mice immunized with VL & VH, VL and reopeptide all developed type specific neutralizing antisera to reovirus type 3, whereas control peptides were unable to do so. In addition, the peptides derived from the anti-receptor antibody were able to stimulate in vivo delayed type hypersensitivity responses that were specific for reovirus containing the type 3 hemagglutinin. Therefore, we have been able to demonstrate that the epitope defined by sequence homology between an anti-receptor antibody and the viral hemagglutinin clearly defines the neutralizing epitope of the hemagglutinin. This approach should allow for the rapid identification of functionally important epitopes for the development of vaccines.

OP8.10

ASPECTS OF THE MOLECULAR INTERACTIONS BETWEEN INFLUENZA NEURAMINIDASE AND ANTI-NEURAMINIDASE ANTIBODIES.

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The three dimensional structure of a complex consisting of an Fab fragment of monoclonal antibody NC41 bound to the viral antigen, influenza A virus neuraminidase (NA) subtype N9, has been solved at 3 A resolution (P.M. Colman et al, submitted). The x-ray diffraction analysis of this complex shows an interaction between four surface loops comprising the epitope of the NA and the variable region of the antibody. Interestingly, one of the epitope surface loops on the NA has been distorted. To further investigate the nature of the interactions, the effects of several mutations in the antigen and antibody molecules, generated by oligonucleotide-directed site-specific mutagenesis or occuring naturally and selected by cell sorting, have been studied.

OP8.11

NEUTRALIZING IgG INHIBITS SECONDARY (BUT NOT PRIMARY) UNCOATING OF INFLUENZA VIRUS. R. Rigg and N. J. Dimmock. Department of Biological Sciences, University of Warwick, Coventry, CV4 7AL, U.K.

Previous studies have demonstrated that influenza virus neutralized by monoclonal or polyclonal anti-HA IgG's do not hinder virus attachment or entry into BHK or CEF cells. Since the lipid envelope of such virus remains cytoplasmic while the viral RNA and associated proteins become nuclear it is concluded that the initial uncoating process is also unaffected. However we can detect no primary transcription in cells inoculated with neutralized virus. In order to define the defect responsible for neutralization we have investigated RNase sensitivity of the viral genome as a measure of the availability of the genome for transcription (defined here as 'secondary uncoating'). We find that the genome of neutralized virus remains resistant to RNase while the majority (upto 60%) of the genome of infectious virus is degraded. These data support our hypothesis that neutralizing antibody (anti-HA) on the outside of the virus particle is able to influence events taking place in its interior.

OP8.12

Recognition of Epitopes on Bovine Herpesvirus Type 1 Glycoproteins by Bovine Antibodies. J.K. Collins, V.K. Ayers, J. Carman. Diagnostic Laboratory, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO 80523.

A panel of monoclonal antibodies (MCAs) to BHV-1 was used to examine the immune response to the virus in naturally exposed and experimentally infected cattle. Sera from experimentally infected calves were analyzed in a competition ELISA. Antibody to an epitope on gp-GE appeared as early as 4 days post infection, although virus-neutralizing antibody did not appear until day 8 or later. Antibody production peaked at around 13 to 18 days post infection but the level of antibody to each epitope varied. Competition by sera from cows naturally exposed to BHV-1 was compared to virus neutralization titer. Competition with most of the MCAs correlated linearly with neutralization titers. However, competition with 2 of the MCAs was maximal even at the lowest neutralization titer, and competition with another MCA, specific for a non-glycosylated core protein, was negative. This was in contrast to the high level of competition with this core protein-specific MCA seen in sera from the experimentally-infected calves. Selected sera from the naturally exposed group were also examined by radioimmunoprecipitation (RIP). The relative levels of glycoproteins that were precipitated did not always correlate with the levels of competition for epitopes on these glycoproteins. These results may be useful in elucidating which epitopes on the virus are important to the production of protective antibodies.

OP9.1

FOWLPOX VIRUS: ITS STRUCTURAL PROTEINS AND IMMUNOGENS AND THE DETECTION OF VIRAL-SPECIFIC ANTIBODIES BY ELISA. A. Deuter, A. P. A. Mockett, D. J. Southee, F. M. Tomley. AFRC Institute for Animal Disease Research, Houghton Laboratory, Houghton, Huntingdon, Cambs. PE17 2DA, UK.

Fowlpox is a slow-spreading viral disease of chickens characterized by cutaneous lesions on the unfeathered skin of the head, neck, legs and feet and/or by diphtheritic lesions in the upper digestive and respiratory tract.

Work was undertaken to study the virus structure. A possible 30 structural polypeptides in the fowlpox virion were identified. There were three major structural proteins with molecular weights of 91, 64 and 58K daltons which were not major immunogens, as assayed using the immunoblot technique. Antibodies in chicken sera taken after a primary fowlpox inoculation reacted strongly only with polypeptides of 37 and 35K daltons, whereas antibodies in sera taken after a secondary inoculation reacted strongly with a number (six) of other viral structural polypeptides. The location in the virion and function of the viral polypeptides has yet to be ascertained. However, preliminary studies using the Chloramine T method to label the virus with 125-I have shown that there are at least four to six external proteins. In addition, an ELISA was developed for the detection of antibodies to fowlpox virus. The primary and secondary IgG humoral antibody response was determined.

OP9.2

COMPARISON OF THE GENOME ORGANISATION OF FOWLPOXVIRUS WITH VACCINIA VIRUS. F. M. Tomley, M. M. Binns, M. E. G. Boursnell, J. I. A. Campbell. AFRC Institute for Animal Disease Research, Houghton Laboratory, Houghton, Huntingdon, Cambs. PE17 2DA, UK.

Fowlpoxvirus, an avipoxvirus, contains a genome of approximately 160 x 10^6 Daltons which is almost 50% bigger than that of the orthopoxvirus vaccinia. Restriction enzyme cleavage patterns of DNA from the two viruses show no relatedness and the DNAs do not cross hybridise under normal conditions of stringency. We have identified a number of genes in fowlpoxvirus which are homologous to vaccinia virus genes and, by locating the map position of these genes on the fowlpoxvirus genome, will compare the physical organisation of these two virus genomes. The amount of sequence divergence of some genes conserved between the two viruses will be discussed.

OP9.3

INTERMEDIATE FILAMENTS ARE INVOLVED IN FROG VIRUS 3 ASSEMBLY. K. G. Murti, R. Goorha, M. W. Klymkowsky. Department of Virology and Molecular Biology, St. Jude Children's Research Hospital, Memphis, Tennessee 38101-0318, U.S.A.

Frog virus 3 (FV3) assembles in morphologically distinct assembly sites in the cytoplasm. Early in infection the intermediate filaments reorganize, delimit the virus assembly sites, and remain so throughout infection. To study the role of intermediate filaments in FV3 assembly site formation, we used taxol or intracellular injection of antivimentin antibodies to disrupt the intermediate filament organization prior to FV3 infection.

Immunofluorescence and electron microscopic studies revealed that in both taxol-treated or antivimentin-injected cells FV3 formed abnormal assembly sites. The sites 1) were not encircled by intermediate filaments, 2) infiltrated by cell components, and 3) contained fewer assembling virions than in controls. The results show that intermediate filaments play an important role in maintaining the structural integrity of FV3 assembly sites which in turn is essential for an efficient assembly of FV3.

OP9.4

TUMORIGENIC POXVIRUSES SHOPE FIBROMA VIRUS AND MYXOMA VIRUS POSSESS GENES RELATED TO EPIDERMAL GROWTH FACTOR. C. Upton, J. Macen and G. McFadden. Dept. of Biochemistry, University of Alberta, Edmonton, Canada.

Although poxvirus replication and assembly occur outside the host cell nucleus within virosomes found in the cytoplasm of infected cells, a number of poxviruses are known to be responsible for proliferative diseases. Examples of such tumorigenic poxviruses are Shope fibroma virus (SFV) which induces benign fibromas in rabbits and Molluscum contagiosum which causes benign tumor-like epidermal lesions in man. Vaccinia virus, a cytolytic *orthopoxvirus*, and SFV, a tumorigenic *leporipoxvirus* , both encode gene products, designated vaccinia growth factor (VGF) and Shope fibroma growth factor (SFGF) respectively, which share significant amino acid homology with epidermal growth factor (EGF) and transforming growth factor alpha. The role(s) of such growth factors in poxviral replication and virus-specific cytopathology is still undefined, but two possibilities are that such gene products could [1] facilitate S phase independence of viral replication by stimulating cellular functions such as nucleotide pool levels and ribosome availability, and [2] mediate the cellular proliferation often observed concomitantly with poxviral infections, albeit to markedly different extents with different poxviruses. Poxviral growth factors may play a central role in virus/cell interactions since the genome of malignant rabbit virus (MRV), a natural recombinant between SFV and myxoma virus, induces SFV-like fibromas during the initial stages of infection of domestic rabbits. MRV contains only a few kb of SFV DNA sequences with the replacement of a comparable extent of myxoma virus sequences. This region of the SFV genome includes four intact major open reading frames plus the intact SFGF gene. Because of the unusual pathology of MRV infections it was of interest to determine whether that myxoma virus also possesses an EGF-like growth factor gene and examine its relationship to SFGF and VGF.

OP9.5

THE ROLE OF THE SHOPE FIBROMA GROWTH FACTOR IN THE PROLIFERATION OF SHOPE FIBROMA VIRUS INFECTED CELLS. C. Macaulay, A. Opgenorth, C. Upton, J.L. Macen, and G. McFadden. Dept. of Biochemistry, University of Alberta, Edmonton, Alberta, Canada, T6G 2H7.

Shope Fibroma Virus (SFV) is a tumorigenic *Leporipoxvirus* which causes benign fibromas in adult rabbits and invasive fibrosarcomas in immunocompromised and newborn rabbits. Like other poxviruses SFV replicates in the cytoplasm of infected cells but unlike the cytolytic *Orthopoxvirus* vaccinia, viral infection in tissue culture causes cellular proliferation. Both Vaccinia and SFV contain a gene (VGF and SFGF respectively) with a significant degree of amino acid homology to epidermal growth factor. The role of this growth factor related gene plays in the proliferative response of SFV-infected cells is being investigated by examining SFGF expression in SFV infected cells and in uninfected cells stably transformed with the SFGF gene expressed under the control of the SV40 promoter.

OP9.6

THE MORPHOLOGY AND MORPHOGENESIS OF A FROG ERYTHROCYTIC VIRUS. J. Gruia-Gray, M. Petric, S. S. Desser. Department of Zoology, University of Toronto, Toronto, Canada.

The Frog Erythrocytic Virus (FEV) is an enveloped, icosahedral DNA virus that occurs in the cytoplasm of erythrocytes of bullfrogs (Rana catesbeiana), green frogs (Rana clamitans) and mink frogs (Rana septentrionalis) from Algonquin Park, Ontario (lat. 45°35'N, long. 78°30'W). The virus, which has been designated as a member of the family Iridoviridae measures 370-450nm in diameter, making it one of the largest icosahedral viruses known to date. It is composed of at least 11 structural proteins ranging in molecular weight from 36 to 101 kDa. During morphogenesis, the virus buds from the periphery of the viroplasm and sequesters a portion of its contents to form a complete nucleocapsid. The naked virus particle appears to acquire an envelope from lamellar membranes elaborated within the erythrocyte cytoplasm.

OP9.7

IN VITRO TRANSCRIPTION OF AFRICAN SWINE FEVER VIRUS DNA BY A SOLUBLE EXTRACT FROM INFECTED CELLS. F. Caeiro and J.V. Costa. Gulbenkian Institute of Science, Oeiras, Portugal.

An in vitro transcription system was prepared from the cytoplasmic fraction of Vero cells infected with African swine fever virus and treated with lysolecithin. This system can transcribe the endogenous viral DNA either early or late in infection and in vitro transcribed RNAs are translatable. The extracts are also active in in vitro translation. They can also be made dependent on exogenous DNA templates after treatment with micrococcal nuclease. In vitro transcription by this system is insensitive to α-aranitin.

OP9.8

AFRICAN SWINE FEVER VIRUS-SPECIFIC DNA-BINDING PROTEINS. A. Esteves, G. Ribeiro and J.V. Costa. Gulbenkian Institute of Science, Oeiras. Portugal.

DNA-binding proteins (DBP) induced by African swine fever virus in infected Vero cells were identified by SDS-PAGE and two-dimensional electrophoresis of proteins separated by chromatography on DNA-cellulose or by binding of nick-translated DNA to Western blots of infected cell protein. Both native and denatured DNA were used. Twenty virus-specific DBP were detected, most of them in NEPHGE gels for basic proteins. Seven of these proteins are structural proteins. Twelve are late proteins, the remaining being early proteins synthesized before viral DNA replication. Most of the virus-specific DBP bind both to double-stranded and to single-stranded DNA, although in some cases with different affinities. Three DBP bind only to single-stranded DNA and only one binds exclusively to native DNA.

Fractions eluted from DNA-cellulose columns were assayed for enzymatic activities. The virus-induced single-stranded-DNA nuclease and RNA polymerase did not bind to DNA. In contrast, the viral DNA polymerase binds strongly to DNA.

OP9.9

ON THE ICOSAHEDRAL STRUCTURE OF LYMPHOCYSTIS DISEASE VIRUS (LDV), A FISH IRIDOVIRUS. L. Berthiaume*, R. Alain*, J. Robin**. (*) Centre de recherche en virologie, Institut Armand-Frappier, Université du Québec, Laval, Qué., Canada, and (**) Département de biologie, Faculté des sciences, Université de Sherbrooke, Sherbrooke, Qué., Canada.

LDV is a large fish iridovirus, about 200 nm in diameter, which replicates in the cytoplasm, but does not acquire an extra envelope by budding through the cytoplasmic membrane. While the virus shows a typical icosahedral symmetry on ultrathin sections, some negative staining observations are inconsistent with this interpretation. Indeed, some virus particles show a capsid-like structure with an hexagonal outline and sharp edges, and with surface subunits in a regular 60° lattice, suggesting an icosahedral structure. However, other virus particles show a tendency to swell and become spherical; the inner membrane underneath subunits sometimes ruptures, revealing curved edges, and surface subunits occasionally appear in 90° lattice. These features do not conform with true capsomer arrangement. One explanation for the relative flexibility of the capsid could be related to the presence of lipids in its inner structure. It appears that a distinction should be established between rigid capsid formed solely with proteins, like typical icosahedral viruses, and flexible capsid with a lipoproteic layer in its inner structure as for the iridoviruses.

OP9.10

EFFECT OF DETERGENTS AND PHOSPHOLIPASES ON THE STRUCTURE OF AFRICAN SWINE FEVER VIRUS (ASFV). G. Schloer and L. Berthiaume. Plum Island Animal Disease Center, USDA, ARS, Greenport, NY and Centre de Recherche en Virologie, Institut Armand-Frappier, Universite du Quebec, Laval, Quebec,Canada.

African swine fever virus (ASFV) is a large DNA virus, which has been classified as an Iridovirus. The virus replicates in the cytoplasm and acquires an outer membrane by budding from the infected cell membrane. A double row of subunits projecting from the surface of the virus are seen in electron micrographs of the virus. A loose irregular membrane, the envelope, surrounds the subunits. Subunits are seen in disrupted virions. Octyl glucoside treatment of the virus rapidly removes the surface exposing a spherical core-like structure. Phospholipase C treatment of extracellular virus results in the disaggregation of the outer subunits revealing an inner structure, the nucleocapsid, 154 x 180 nm in size containing a central oblong core, 30 x 70 nm in size. Phospholipase A slowly removes the subunits revealing a smooth hexagonal structure. The results from the micrographs indicate that ASFV has an inner nucleocapsid, and an outer capsid-like structure of subunits embedded in a lipid membrane. Both structures may be involved in determining the icosahedral shape of the virus.

OP9.11

METHYLATION OF THE PROMOTER OF AN IMMEDIATE-EARLY FROG VIRUS 3 GENE DOES NOT INHIBIT TRANSCRIPTION. J. P. Thompson, A. Granoff, D. B. Willis. Department of Virology and Molecular Biology, St. Jude Children's Research Hospital, Memphis, Tennessee, USA.

Methylation of critical sites within the promoter region of a gene inhibits transcription by RNA polymerase II. The genome of the iridovirus frog virus 3 (FV3) is highly methylated, yet this virus uses RNA polymerase II for at least the immediate-early stage of transcription. We have previously shown that an FV3-induced trans-acting protein alters either host RNA polymerase II or methylated template to allow transcription from methylated promoters. Since immediate-early genes are transcribed in the absence of de novo protein synthesis, it appears that the virus-induced protein is not required for transcription of this class of genes. Genomic sequencing of an immediate-early gene of FV3 revealed that the promoter of this gene is methylated. Transient expression assays, using a clone of this gene, indicated that methylation of the promoter does not inhibit transcription by RNA polymerase II. These findings are consistent with the hypothesis that the immediate-early genes of FV3 are not inhibited by methylation, and that genes inhibited by methylation are transcribed only after expression of the virus-induced trans-acting protein.

OP9.12

A MODEL VIRUS-CELL SYSTEM TO STUDY THE PERSISTENCE OF AFRICAN SWINE FEVER VIRUS. Dolores G. Villalón and Carmen Gil-Fernández. Centro de Investigaciones Biológicas. Velázquez, 144. 28006 Madrid. SPAIN

Vero cells growing on monolayer cultures were infected with African swine fever virus and treated with different concentrations (100, 50 and 10 ug/ml) of 5-iodo-2'-deoxyuridine. Some of these cultures developed a persistent infection with cycles of virus production,after 12 cell passages they were cured and no virus could be detected.

These cells have been maintained for more than two years and they show resistance to superinfection with African swine fever virus, herpes simplex type 1 and vaccinia virus. In the case of ASF virus, when the cells were reinfected with wild type virus a persistent-state was established in the absence of any drug treatment. We have developed an approach to the study of viral persistence which occured in natural infected pigs within endemic areas.

OP9.13

COMPARATIVE IMMUNITY OF FOWL POX VACCINES.II. EFFECT OF AFLATOXIN B_1 ON THE IMMUNITY TO FOWL POX VACCINE. D.K.Sarma, S.N.Sharma, Department of Vety Bacteriology & Virology, Punjab Agricultural University,Ludhiana-141 004, India.

The comparative immunity of five fowl pox virus vaccines was studied by four different routes,viz.intramuscular (I/M), feather follicle (F/F), oral (drinking water) and intranasal (I/N) routes in chicks of different age group. Of the five vaccines, the vaccines such as IVRI,BM and Srini are the commercially available fowl pox vaccines in India. Besides, the HP_1 strain of fowl pox virus obtained through courtesy of Prof (Dr) A.Mayr, West Germany and a locally isolated fowl pox virus strain were employed in the immunity trials. All the vaccines virus wore adapted on the chorioallantoic membrane of 12 days embryonated eggs and the virus titer of $10^{6.09}$, $10^{6.36}$, $10^{6.39}$ egg infective dose 50(EID_{50}) respectively for IVRI, BM and Srini vaccines and $10^{6.39}$, EID_{50} for HP_1 and local strains were used for immunization irrespective of the routes. Vaccination by I/M and F/F routes was done at 8th week of age and revaccination at 14th week. First vaccination by oral and I/N routes was done at 3rd week followed by second vaccination at 7th week of age. Immune status of the vaccinated birds was assessed by various serological and challenge tests.

Trial II was conducted to study the effect of aflatoxin B_1 (0.25 µg/g) on the immunity to fowl pox vaccine.

The detailed results were presented and discussed.

OP9.14

EXPRESSION AND REGULATION OF VACCINIA VIRUS GENES. C. Edbauer, J. Miner, D. Hruby. Center for Gene Research, Department of Microbiology, Oregon State University, Corvallis, Oregon, USA, 97331-3804

The molecular mechanisms that are responsible for the expression and regulation of vaccinia virus (VV) genes remain an enigma. In view of the amount of work which has been necessary to generate our present level of understanding how genes are regulated in other eukaryotic systems, this is not likely to be a trivial task. One suspects that the answers will involve RNA polymerase modifications, cis-recognition signals, and trans-acting regulatory proteins. Given the complexity of the situation, it has been our lab's prejudice that a reasonable approach is to identify a few VV genes of interest and study them in detail with regard to these questions. In the collection of VV genes under current investigation in our laboratory we have a bonafide early gene (thymidine kinase), an early gene whose activity is essential late in infection (α-amanitin resistance), a delayed-early gene involved in the switch between early and late modes of gene expression (ts17), and a number of differentially-regulated late genes (Hind III D/A cluster). It is hoped that by identifying the regulatory elements of some or all of these genes we will be able to recognize the common and unique features of each. This information should provide considerable insight into the overall regulatory strategy employed by VV.

OP9.15

DNA DELETIONS AND ADDITIONS IN AFRICAN SWINE FEVER VIRUS (ASFV) STRAIN E-70 AFTER ADAPTATION TO GROWTH IN THE MS MONKEY KIDNEY CELL LINE.

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Physical maps for the fragments produced by Cla I,Sal I and Sma I have been constructed for the DNAs of ASFV growing in pig leukocytes (strain E70L6) and after adaptation to MS cells (strain F70MS14).The mapping data revealed that the size of ASFV DNA strain E70L6 was 173 Kbp while after adaptation to MS cells was reduced to 156 Kbp.The size diminution was produced by deletions and additions mainly in the terminal regions of the genome After fourty four passages in MS cells, additional deletions and size gains in specific viral locations were detected.

OP9.16

GENETIC AND PHYSICAL MAP OF THE VACCINIA VIRUS HIND III D FRAGMENT. Edward G. Niles, J. Lee-Chen, J. Seto, R. C. Condit. Department of Biochemistry. SUNY at Buffalo, Buffalo, New York 14214, USA.

The Vaccinia Virus Hind III D Fragment is 16,060 base pairs in length (Niles et al, Virology. 153, 96, 1986; Weinrick, S. and Hruby, D. Nuc. Acids Research. 14, 3003, 1985) and contains 13 complete open reading frames. Marker rescue of ts mutants and complementation analysis has sorted 17 ts mutants into 7 complementation groups which map in the Hind III D fragment. We have refined the genetic map so that each mutant in each complementation group has been unequivocably assigned to a single gene. Furthermore, we have carried out transcript mapping studies for each gene. Of the 13 genes within the Hind III D fragment, 5 are expressed early after infection, 5 are expressed only at late times, and three are expressed both early and late. The transcription start sites have been identified for 5 early, 3 late and 2 early plus late genes. The precise 3' ends have been located in 3 early genes and 2 early transcripts in 2 early plus late genes.

The overall arrangement of the genes in the Hind III D fragment minimizes conflicts which might occur during the expression of the closely packed adjacent genes.

OP9.17

A NUCLEOTIDE SEQUENCE OF GENOME-ASSOCIATED SMALL RNA EXISTING IN A VACCINIA VIRUS PARTICLE. T. Urushibara, T. Okamoto, C. Nishimura[1], K. Kohno & K. Miura. School of Pharmaceutical Sciences, Kitasato University[1], Tokyo, and Faculty of Engineering, University of Tokyo[2], Tokyo, Japan.

Vaccinia Virus (VV) is well known as that contains a double-stranded DNA genome of MW = 1.6×10^8. Planterose et al. (1962) first indicated that small amounts of RNA coexisted in VV particles besides viral DNA genome. In the previous Congress at Sendai (1984), we demonstrated that this small RNA was separable from viral DNA genome when carefully extracted from purified cores of VV. Then, molecular class of this RNA was determined to be 4S by sucrose gradient centrifugation, Sephadex G-25 chromatography and polyacrylamide gel electrophoresis.

Recently, specific labeling of VV-associated RNA at the 3'-terminus has come to easy with success by using T4 ligase and ^{32}pCp in our laboratories. Then we determined nucleotide sequence of this RNA by limitting enzymatic digestion. As a result, we have found that VV-associated small RNA consisted of 80 nucleotides with cytosine at 3'-terminus. Detections of a few modified bases and intramolecular double-stranded structures indicated that it has substantially a tRNA-like structure. The nucleotide sequence and secondary structure of VV-associated RNA will be discussed.

OP9.18

TRANSCRIPTS OF A STRONGLY-EXPRESSED LATE GENE OF CDWPOX VIRUS HAVE POLY(A) LEADER SEQUENCES. D. Patel and D. J. Pickup. Department of Microbiology and Immunology, Duke University Medical Center, Durham, NC 27710 USA.

The major component of the A-type inclusions produced in cells infected with cowpox virus (CPV) is a 160 kDa protein. Late in the infection this is one of the most abundant proteins in the cell. The gene (160K gene) encoding this protein is located in the Kpn G fragment of the DNA of CPV (Brighton Red strain). The nucleotide sequence of the DNA flanking the translational initiation codon of this gene has been determined. A 21 nucleotide sequence immediately upstream of the initiation codon consists entirely of A and T residues.

S1-nuclease protection experiments, primer-extension experiments, and nucleotide sequence analysis of both RNA and cDNA have been used to characterize the 5'-ends of the transcripts of the 160K gene. These analyses showed that these transcripts have multiple 5'-ends. Furthermore, the 5'-ends of most of these transcripts contain untranslated leader-sequences consisting of from 5 to 21 A-residues. Interestingly, these poly(A) leaders are not complementary to the corresponding sequence immediately upstream of the initiation codon in the template strand of the 160K gene.

The biological significance of the poly(A) leaders is under investigation.

OP9.19

IMMUNOCHEMICAL CHARACTERIZATION OF VIRUS ANTIGENS EMBEDDED IN THE SURFACE OF VACCINIA-INFECTED CELLS. BARRY ZIOLA[1], A SALMI[2], R MARUSYK[2]. [1]Immunovirology Research Group, Dept. of Micro., Univ. of Saskatchewan, Saskatoon, Sask., Canada; [2]Viral Pathogenesis Research Unit, Dept. of Med. Micro. & Infectious Dis., Univ. of Alberta, Edmonton, Alta., Canada.

Given potential usage of vaccinia virus (VV) as vaccine vector for other human viruses, it is important to delineate which virus antigens are involved in immune responses controlling spread of VV. One aspect of this means determining which VV antigens are in the plasma membrane, such that VV-infected cells can be killed by antibody and/or cell-mediated mechanisms.

With 25 monoclonal antibodies (mabs) specific for the surface of VV-infected cells, as well as polyvalent human and rabbit anti-VV sera, we have determined there are 5 major VV antigens in the outer membrane of VV-infected cells. Molecular weights of the proteins are 89,000 (89K), 62K, 42K, 37K and 34K. Hemagglutination inhibition with the mabs confirmed the 89K protein is the VV hemagglutinin.

Through mab immunosorbent isolation of each of these proteins, it will now be possible to obtain the polyvalent antisera needed to ascertain which of the 5 VV proteins are involved in antibody-mediated killing of VV-infected cells.

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OP9.20

RAPID SELECTION OF RECOMBINANT VIRUSES: EXPRESSION OF BETA-GALACTOSIDASE BY USING VACCINIA VIRUS VECTORS. C. Huang, A. Grzelecki, and D. Panicali*, Wadsworth Center for Laboratories and Research, New York State Department of Health, Albany, New York, U.S.A. *Applied bioTechnology Inc., 80 Roger Street, Cambridge, MA, U.S.A.

Plasmids were constructed fusing vaccinia transcriptional regulatory sequences (promoters) to the lacZ gene of Escherichia coli. These recombinant plasmids were used to compare relative promoter strengths, to identify the essential region of the transcriptional unit, and to construct recombinant vaccinia virus producing β-galactosidase (βGal). Viruses synthesizing βGal were determined by utilizing the chromogenic substrate, X-gal, to form blue plaques. A recombinant virus producing βGal was then used to select a second recombinant virus. This was accomplished via in vivo recombination replacing the lacZ gene with a coding sequence of the gene of interest. The recombinant virus was selected by its inability to form blue plaques under appropriate conditions.

OP9.21

CHARACTERIZATION OF THE REPETITIVE DNA SEQUENCES IN THE GENOME OF INSECT IRIDESCENT VIRUS TYPE 6 BY MOLECULAR CLONING AND PHYSICAL MAPPING. M.Fischer, J.B.Soltau, P.Schnitzler, H.Delius*, G.Darai. Institute of Medical Virology of the University of Heidelberg; *European Molecular Biology Laboratory, Heidelberg, Federal Republic of Germany.

The genome of Insect Iridescent Virus Type 6 - Chilo Iridescent Virus (CIV) - was shown to be circularly permutated and terminally redundant. A defined and complete gene library of the whole CIV DNA sequences (209 kbp) was established. The physical map of the viral genome was constructed for the restriction endonucleases ApaI, Asp718, BamHI, EcoRI, NcoI, PvuII, SalI, SmaI, and SphI.

DNA-DNA hybridization experiments and heteroduplex mapping revealed the presence of repetitive DNA elements in the viral genome, which are located in the EcoRI DNA fragment H (9.8 kbp; 0.535-0.582 mu) and in the EcoRI DNA fragment C (13.5 kbp; 0.909-0.974 mu). The further characterization of these repetitive DNA elements by fine mapping and nucleotide sequencing is necessary for the understanding of the functional activity and regulatory role of these elements.

DFG grant Da 142/2-2

OP9.22

IDENTIFICATION AND NUCLEOTIDE SEQUENCE ANALYSIS OF THE REPETITIVE DNA ELEMENTS IN THE GENOME OF FISH LYMPHOCYSTIS DISEASE VIRUS. P.Schnitzler, H.Delius*, J.Scholz, M.Touray, E.Orth, G.Darai. Institute of Medical Virology of the University of Heidelberg; *European Molecular Biology Laboratory, Heidelberg, Federal Republic of Germany.

The genome structure of the Fish Lymphocystis Disease Virus (FLDV) was found to be circularly mutated and terminally redundant. A defined gene library corresponding to 100% of the DNA sequences of the viral genome (98 kbp) was established. Using this gene library, DNA-DNA hybridization experiments and heteroduplex mapping two repetitive DNA elements were found at the coordinates 0.034 to 0.165 mu (a part of the Eco RI DNA fragment B; 12.7 kbp, 0.034 to 0.168 mu) and at the coordinates 0.718 to 0.736 mu (Eco RI DNA fragment M; 1.4 kbp). The nucleotide DNA sequences of the repetitive DNA element were determined and compared to the repetitive DNA sequences of different origin. Further analyses revealed that these repetitive DNA elements are conserved in the genome of FLDV obtained from different pleuronectes, indicating that these DNA sequences play a functional role in virus progeny, e.g. virus replication and encapsidation.

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OP9.23

CHARACTERIZATION OF THE GENOME OF MOLLUSCUM CONTAGIOSUM VIRUS BY MOLECULAR CLONING AND PHYSICAL MAPPING. J.Scholz, J.Bugert, M.Fischer, A.Rösen-Wolff, G.Darai. Institute of Medical Virology of the University of Heidelberg, Federal Republic of Germany.

Molluscum contagiosum Virus (MCV), an unclassified member of the poxviridae family, induces epidermal hyperplasia and benign skin tumours in man. The comparative analysis of the genome of about 100 independently isolated viruses and DNA-DNA hybridization experiments revealed that MCV isolates can be classified into two different types. A defined gene library of MCV DNA sequences of MCV type 1 or 2 was established. Using this defined gene library the physical map of MCV-1 was constructed for the restriction enzymes BamHI and HindIII. The relatedness of the DNA sequences of the MCV genome to the genome of other members of the poxvirus family was investigated. This study is necessary to identify the evolutionary relationship of MCV in the family poxviridae and could help to resolve epidemiologic questions of this disease.

This study was supported by the Förderverein zur Bekämpfung der Viruskrankheiten e.V.

OP10.1

MONOCLONAL ANTIBODIES REVEAL A SPECIFIC ASSOCIATION BETWEEN THE M PROTEIN OF NEWCASTLE DISEASE VIRUS AND LIPOSOMES. K.S. Faaberg and M.E. Peeples. Department of Immunology/Microbiology, Rush University, Chicago, IL, USA.

The matrix (M) protein of Newcastle disease virus, a paramyxovirus, associates with liposomes of positive, negative, or neutral charge. Topological mapping has revealed that the M protein has at least 3 discrete antigenic sites (M1, M2, M3) recognized by 3 sets of monoclonal antibodies.

One antibody from each set was selected to study the association between the M protein and liposomes. Positive, negative, and neutral liposomes were prepared. All three monoclonal antibodies were able to bind to affinity purified, monomeric M protein after it was attached to positive or neutral liposomes. However, none of the antibodies were able to bind when the M protein was attached to negatively charged liposomes. This result suggests a specific orientation of the M protein in its interaction with liposomes. In the reverse experiment, antibodies were incubated with M protein before adding liposomes. None of the antibodies were able to block the interaction. This result suggests that these antigenic sites are not directly involved in the binding of the M protein to liposomes. The M protein appears to associate with positive and neutral liposomes in a manner which does not involve antigenic sites M1, M2 and M3. The M protein appears to associate with negatively charged liposomes in a different manner.

OP10.2

CHARACTERIZATION AND CLONING OF BOVINE RESPIRATORY SYNCYTIAL VIRUS. R.A. Lerch[1], E.J. Stott[2] and G.W. Wertz[1]. [1]Department of Microbiology, The University of Alabama at Birmingham, Birmingham, AL 35294 [2]Department of Microbiology, I.R.A.D. Compton, UK.

The virus specific proteins and mRNAs of Bovine Respiratory Syncytial virus (BRSV) were compared by gel electrophoresis to the virus specific proteins and mRNAs of the A2 strain of Human Respiratory Syncytial virus (HRSV). Both viruses were grown in bovine nasal turbinate cell monolayers. Radioactively labeled proteins, or mRNAs were extracted from infected cell monolayers and analyzed by either SDS polyacrylamide (proteins) or agarose-urea (RNA) gel electrophoresis. Migrational differences were observed in the presumed fusion protein, F, of BRSV, and in the presumed G, P, 1A, 1B, 1C, and F protein coding mRNAs of BRSV.

To further analyse the BRSV mRNAs, cDNAs were synthesized by strand replacement synthesis as described by Gubler and Hoffman (1983). BRSV specific cDNAs were distinguished from cDNAs of cellular origin, and grouped by northern blot and dot blot analysis. The characterization of these cDNAs will be presented.

OP10.3

STRUCTURAL REQUIREMENTS FOR THE ANTIGENICITY OF THE HEMAGGLUTININ-NEURAMINIDASE (HN) GLYCOPROTEIN OF NEWCASTLE DISEASE VIRUS (NDV) Bin Gotoh, K. Nishikawa, and Y. Nagai, Research Inst. for Disease Mechanism and Control, Nagoya University School of Medicine, Nagoya 466, Japan.

The HN glycoprotein of NDV is important for the initiation of the infection by mediating the virus attachment to host cell receptor. The glycoprotein has at least four antigenic sites I-IV which are topologically and operationally discriminated from one another by specific monoclonal antibodies. In this study, we examined the cellular compartments and metabolisms for the formation of the antigenic sites. There appeared to be marked site-specific requirements with respect to glycosylation and disulfide bond formation. Antigenic site I is completely independent of glycosylation, whereas the other three sites depend on glycosylation. Disulfide bonds are important for the formation of antigenic sites I,III,IV, while site II requires neither intra- nor intermolecular disulfide bonds. All of these metabolic requirements appeared to be provided within the rough endoplasmic reticulum. The HN genes of the variants selected by each antibody are being sequenced.

OP10.4

RESISTANCE OF ALTERED BHK CELLS TO HVJ (SENDAI VIRUS) INFECTION. T. Miyadai, and Y. Kimura. Department of Microbiology, Fukui Medical School, Fukui, Japan.

Altered BHK cells, designated BHK-R cells, have been passaged in the continuous presence of HVJ. After serial passages in medium free of HVJ, BHK-R (BHK-R1, -R2, -R3, ..) cells gradually become susceptible to the HVJ infection.

By the Scatchard analysis it became clear that a very few binding sites for HVJ were presenct on the surface of BHK-RO, but BHK-R1 cells which still show a distinct resistance to HVJ had already acquired almost equivalent number of binding sites to noumal BHK cells. Phenotypically mixed virus with a VSV genome and a coat of HVJ envelope produced VSV plaques on BHK-R1 cells as effeciently as on normal BHK cells. Neither actinomycin-D resistant RNA synthesis nor HVJ-specific protein synthesis could be detected in BHK-R1 cells infected with HVJ. It is suggested that HVJ growth in BHK-R1 cells is blocked at the level of transcription.

OP10.5

STRUCTURAL AND FUNCTIONAL DIFFERENCES IN THE HN PROTEIN OF ENDERS AND FIELD STRAINS OF SENDAI VIRUS. W. L. Gorman, S. D. Thompson, and A. Portner. Department of Virology and Molecular Biology, St. Jude Children's Research Hospital, Memphis, Tennessee, USA

The HN molecule of Sendai virus is a multifunctional protein involved in virus attachment, hemagglutination, fusion, and neuraminidase activities. Monoclonal antibodies directed aginst the HN of the Enders strain had identified two of four antigenic sites involved in hemagglutination inhibition activity. In contrast, all four antigenic sites were involved in hemagglutination inhibition in viruses isolated from lung tissue of mice that died of pneumonia. Sequence analyses of the HN gene of the Enders and field strains revealed 10 amino acid changes. These results suggest that conformational differences exist between the Enders and field strains and provide an opportunity to learn more about the structure and function of the HN molecule. Comparison of the HN sequence of the field strains with an Enders-derived ts mutant defective in HA and cell attachment suggests which antigenic site may be associated with cell attachment. Studies are underway to further characterize differences in HN protein stability, neuraminidase and hemolysis inhibition, and neutralization between the field and Enders virus strains.

OP10.6

ISOLATION AND CHARACTERIZATION OF A PROTEOLYTICALLY CLEAVED FORM OF THE HN PROTEIN OF SENDAI VIRUS. S. D. Thompson[1], W. G. Laver[2], A. Portner[1]. [1]Department of Virology and Molecular Biology, St. Jude Children's Research Hospital and Department of Microbiology and Immunology, University of Tennessee, Memphis, USA; [2]John Curtin School of Medical Research, Australian National University, Canberra, Australia.

To elucidate the structure of the Sendai virus glycoprotein HN, we have isolated and characterized a soluble form of the molecule. Virions were disrupted using Triton-X 100 and the HN protein was isolated by centrifugation. The deduced primary amino acid sequence of the protein shows only one hydrophobic domain, which is probably responsible for rosette formation in the absence of detergent. This region is located at the amino terminus and is probably the transmembrane anchor region. To avoid rosette formation, the hydrophobic tail was removed by proteolytic digestion. The cleavage site was located at residue 131 by N-terminal amino acid sequence analysis. The cleaved HN retained full neuraminidase activity, indicating that the N-terminal 131 residues are not necessary for enzyme activity. Furthermore, the antigenic structure of HN was not altered by cleavage, since monoclonal antibodies still bound to all four previously defined antigenic sites. Attempts to crystallize this cleaved form of HN are in progress.

OP10.7

CYSTEINE DIFFERENCES MAY ACCOUNT FOR THE EXISTENCE OF MONOMERIC AND DIMERIC HN GLYCOPROTEINS IN VARIOUS NDV STRAINS. J.P. Sheehan, R.J. Syddall, R.L. Glickman, M.A. Bratt and R.M. Iorio. Department of Molecular Genetics and Microbiology, University of Massachusetts Medical School, Worcester, MA, USA.

The Newcastle disease virus serotype demonstrates a wide array of virulence patterns ranging from avirulent to highly virulent strains. A less well-characterized finding is that the hemagglutinin-neuraminidase (HN) glycoprotein of these strains can exist as either a monomer or dimer as defined by SDS-PAGE under non-reducing conditions with apparently no causal relationship between monomeric or dimeric HN and virulence.

From the deduced amino acid sequence of the dimeric HN of strain AV (T. Morrison, personal communication), we have sequenced several other dimeric and monomeric HN's using primer extension and "dideoxy" sequencing. Our preliminary evidence, supported by the published sequences of the monomeric HN's of the Beaudette (Millar et al, J. Gen. Virol. 67, 1917) and B_1-Hitchner (Jorgensen et al, Virology 156, 12) strains, suggests that two of the fourteen cysteine residues present in dimers are substituted in monomers. One residue is internal to the envelope while the second is about 75 residues beyond the membrane spanning region. This suggests the possible involvement of either or both cysteine residues in dimer formation.

OP10.8

BOVINE RESPIRATORY SYNCYTIAL VIRUS FUSION PROTEIN - SPECIFIC MONOCLONAL ANTIBODIES. C.A. Anderson, L.D. Nelson, C.A. Klucas, Department of Veterinary Science, University of Nebraska, Lincoln, Nebraska, USA.

Five hybridomas were produced which secreted monoclonal antibodies (mcAb) to bovine respiratory syncytial virus (RSV). Two antibodies (8G12, 15C7) neutralized the virus and inhibited syncytia formation in vitro. These mcAb also stained, by indirect fluorescent assay, an external envelope protein of living virus-infected cells, and recognized the viral fusion protein (FP) by Western blot analysis of bovine RSV-infected cell lysates. Virus neutralization titers of mcAb 8G12 and 15C7 were equal to or greater than 640. Three other mcAb stained, by indirect fluorescent assay, acetone-fixed virus infected cells but not living cells.

An affinity column was built using mcAb 8G12, which was used to isolate FP from bovine RSV-infected cells. The FP eluted from the column then was used to immunize rabbits. These rabbit polyclonal sera recognized both the intact FP (70K) and the FP subunits (48K and 26K) by Western blot analysis. The sera also neutralized bovine RSV in standard virus neutralization test at 1:24. These results indicate that the FP of bovine RSV is remarkably similar in structure and function to human RSV. Additionally, the FP of bovine RSV is immunogenic and may warrant consideration as a subunit vaccine candidate.

OP10.9

THE POLYPEPTIDES OF MUMPS VIRUS AND THEIR SYNTHESIS IN INFECTED CHICK EMBRYO CELLS. B. H. Wu, Y. C. Li, Department of Virology and Molecular biology, Wuhan University, Wuhan, Hubei, People's Republic of China.

Strain ME of Mumps Virus grow in chick amniotic cavity and purified by differential centrifugation combined with sucrose gradient centrifugation. The structural polypeptides of purified virus were analyzed by SDS polyacrylamide gel electrophoresis. Eleven polypeptides were found. Their molecular weight were between 35K-72K dalton. In addtion, polymers of HN protein and F1 the large subunit of protein were detected. Hela, Vero and Chick embryo (CE) cells were infected with ME strain of mumps virus. The CE cell reveals the most sensitive host cell. CE cell infected with mumps virus and lableed with (^{35}S)-met, SDS-PAGE and examined by autoradiography. It was found at least eight polypeptides were synthesis in the host cells and their molecular weight were between 20.5K-94K dalton. The time course of the synthesis polypeptides inthe cells were studied. Pulse-Chase experiments demonstrated that the posttranlationl procesaion of $F_0 \rightarrow F$.

OP10.10

THE EFFECT OF MEASLES ANTISERUM ON PHOSPHOINOSITIDE METABOLISM IN SSPE INFECTED C6 CELLS

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Treatment of C6 rat glioma cells persistently infected with SSPE virus (C6/SSPE) with measles antiserum results in the loss of detectable expression of all virus proteins. The mechanism of this antigenic modulation is still unknown, but obviously a signal induced by binding of the antibody to the viral membrane antigens is transmitted into the cell.

PIP_2 breakdown was shown to be involved in quite a lot of transmembrane signalling pathways. We looked for the phosphoinositide metabolites in C6/SSPE cells after treatment with measles antiserum. Within 2 minutes PIP_2 was hydrolyzed and there was a significant increase in IP_3, IP_2 and IP_1. The same effect was observed with monoclonal antibody K83 against HA and with FCS. In uninfected C6 cells PIP_2 breakdown can be induced by FCS, but neither by K83 nor by polyclonal antiserum. These results provide a link of phosphoinositide breakdown and antigenic modulation.

This work was supported by Deutsche Forschungsgemeinschaft (SFB 105, C 3).

OP10.11

INFLUENCE OF POLYCLONAL ANTIBODIES AGAINST MEASLES VIRUS ON VIRAL RNA SYNTHESIS IN C6-RAT GLIOMA CELLS PERSISTENTLY INFECTED WITH SSPE VIRUS STRAIN LEC. J. Zinnheimer, K. Koschel, Institute for Virology and Immunobiology, University of Würzburg, Versbacher Str. 7, D-8700 Würzburg, F.R.G.

SSPE is a rare chronic inflammatory disease of the human central nervous system caused by a persistent infection of measles virus. By cultivation of C6-rat glioma cells infected with SSPE virus (C6/SSPE) in presence of measles antibodies a decrease of viral polypeptides in the cell membrane as well as in the cytoplasm occurs. This phenomenon is called antigenic modulation. As previously described the viral mRNA is also diminished after treatment of infected cells with antiserum, but surprisingly the genomic RNA is not influenced.

We investigate whether antigenic modulation affects all infected cells or whether only a few cells escape immunological suppression by antibodies. In situ hybridization studies reveal that all infected cells show a reduction of their viral RNA amount. After removal of antiserum from growth medium a more intense synthesis of viral RNA can be observed.

This work was supported by Deutsche Forschungsgemeinschaft (SFB 105, C 3) and Boehringer Ingelheim Fonds.

OP10.12

CHARACTERIZATION OF, AND PROTECTION WITH MONOCLONAL ANTIBODIES AND T CELL LINES SPECIFIC FOR THE F PROTEIN OF MEASLES VIRUS. P. de Vries, R.S. van Binnendijk, F.G.C.M. UytdeHaag, A.D.M.E. Osterhaus. National Institute of Public Health and Environmental Hygiene, Bilthoven, The Netherlands.

We generated a panel of mouse monoclonal antibodies (MoAb's) and mouse T cell lines with the T helper phenotype directed against the fusion (F) protein of measles virus (MV). Nine anti-F MoAb's proved to recognize at least five partially overlapping antigenic sites on the F protein in a competition assay. None of the anti-F MoAb's exhibited virus neutralizing or hemagglutination inhibiting activity, whereas only one anti-F MoAb (F 3-5) showed hemolysis inhibiting (HLI) activity. Although a polyclonal anti-MV serum and anti-H MoAb's protected mice against experimentally induced MV encephalitis, neither a polyclonal anti-F serum, raised against F iscom nor a pool of the anti-F MoAb's, protected mice. Even the anti-F MoAb (F 3-5) which exhibited HLI activity did not protect the intracerebrally infected mice, although a prolonged survival period was observed. The MV-specific murine T cell lines with the T helper phenotype were generated after immunization of BALB/c mice with F iscom. At least two of them could assist B cells in the production of MV-specific antibodies in vitro. Protection studies in which these cell lines are adoptively transferred to mice are in progress.

OP10.13

ISOLATION AND CHARACTERISATION OF MONOCLONAL ANTIBODIES TO SV5 AND PF-2 VIRUSES. D. Young, R.E. Randall, K.K.A. Goswami, W.C. Russell. Department of Biochemistry and Microbiology, University of St. Andrews, Fife, Scotland.

Although simian virus 5 (SV5) has been shown to infect both dogs and humans, studies on the epidemiology of SV5 infections have been complicated by the evolutionary and antigenic relationship between SV5 and parainfluenza type 2 (PF-2). In order to further characterise the antigenic relationship between these two viruses it was decided to isolate a bank of monoclonal antibodies to SV5 and PF-2.

Fifty four monoclonal antibodies to SV5 and six monoclonal antibodies to PF-2 have been isolated with specificities to the HN, F, M, P and N proteins of SV5 and the HN, N and P proteins of PF-2. Sixteen of these monoclonal antibodies react with the HN protein of SV5 and define at least five different epitopes on this protein. Four of these epitopes are common to human, monkey and dog isolates; the fifth epitope is shared by the human and dog isolates but is not present on the monkey (prototype) isolate. None of the monoclonal antibodies to the HN protein of either SV5 or PF-2 cross react with heterologous virus. The majority of the monoclonal antibodies isolated were specific for the homologous virus. However, two monoclonal antibodies to the M, N or P proteins respectively, were isolated that show cross reactions between SV5 PF-2. None of the monoclonal antibodies cross react with PF-3 or measles virus.

OP10.14

MEASLES VIRUS CLONES CHARACTERIZED BY IN VITRO TRANSCRIPTION, TRANSLATION AND IMMUNOPRECIPITATION. Karl W. Hasel, Peter A. Greer and Stewart Millward. Dept. of Biochemistry, McGill University, Montreal, Canada.

A cDNA library was constructed from poly(A)$^+$ RNA prepared from VERO cells infected with the Edmonston strain of measles virus and five classes of clones were isolated. To assess the coding capacitity of these cDNA clones they were inserted into pSP64, RNA transcribed in vitro, then translated in reticulocyte lysates and immunoprecipitated with specific antisera. This analysis found that the NP, P/C and HA "mRNAs" consistently produced smaller polypeptides that appear to be initiated at internal AUGs. The unglycosylated F protein was found to migrate at a molecular weight of 55KDa.Also an increase in translational efficiency of the F transcripts was achieved by removing 450 bases of the G-C rich 5' non coding region. Probes were prepared from these 5 clones and used to map bicistronic RNA transcripts found in low abundance in measles virus infected cells. A gene order for measles virus of 3'..NP-P/C-M-F-HA-(L)..5' was deduced.

OP10.15

CHARACTERISATION OF ANTIBODIES TO RESPIRATORY SYNCYTIAL VIRUS IN HUMAN SERA. HB Gimenez, HM Keir, MAJ Moffat and P Cash. Departments of Biochemistry and Bacteriology, University of Aberdeen, Aberdeen, Scotland.

Human respiratory syncytial (RS) virus is an important cause of infection of young children. The protein specificities of human antibodies, induced by RS virus, have been determined by immunoblotting. We have analysed serial serum samples from a patient collected over a period of 5 years; the serum CF titres declined from 1:2048 to 1:256 and the neutralising titre decayed from 1:8000 to 1:2300. Immunoblot analysis of the sera showed that antibodies to all the major virus proteins decayed with time but antibodies to VPN41 and VGP48 were affected the least.

We have examined the influence of human sera with RS virus antibodies on the infection of macrophages by RS virus. The sera increased the uptake of virus by the macrophages as determined by immunofluorescent staining. With paired acute and covalescent sera, only the latter caused increased virus uptake. Virus antigen was detected in macrophages for 4 days post-infection. Whether this represented uptake of the initial virus inoculum or virus replication in the macrophages is under investigation.

OP10.16

ANTIGENIC CHARACTERIZATION OF HEMAGGLUTININ-NEURAMINIDASE(HN) ANTIGEN OF HUMAN AND ANIMAL PARAMYXOVIRUSES. M. Matsumoto, K. Nerome, M. Ishida, A. Oya. Department of Virology and Rikettsiology, National Institute of Health, Tokyo, Japan.

Unlike orthomyxovirus which is systematically classified on the basis of antigenic character of its inner and surface glycoproteins, the immunological properties of the viruses belonging to the genus paramyxovirus are not well understood.

The present study was designed to reveal the antigenic relationship amongst HN protein of human and animal paramyxoviruses by using antisera monospecific for the isolated HN subunit. Hemagglutination-inhibition(HI) and neutralization(NT) tests showed that human parainfluenza type 1(P1), type 3 (P3) and Sendai viruses possessed HN antigens related to each other. In the present study, an antigenic determinant sites of P1 virus HN which reacts with anti-P3 serum were found to present inside the molecule. Antiserum monospecific for HN of parainfluenza 4A and 4B viruses cross-inhibited the plaque formation by both viruses, indicating the presence of common antigen. In addition, Mumps virus appeared in NT tests with antiserum to HN to be related to P3 virus. A total of 9 antisera monospecific for HN could differentiate antigenic relatedness between human and animal paramyoviruses.

OP10.17

MOLECULAR CLONING AND NUCLEOTIDE SEQUENCE ANALYSIS OF NEWCASTLE DISEASE VIRUS STRAIN ULSTER

M. S. Millar, P. Chambers and P. T. Emmerson.
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We have reported previously the molecular cloning of Newcastle disease virus (NDV) strain Beaudette C and have determined the nucleotide sequence and deduced amino acid sequence of the HN, F, M and L genes.

We have now constructed a bank of cDNA clones derived from genomic RNA of NDV strain Ulster. Unlike Beaudette C, strain Ulster is extremely avirulent. The sequence data obtained so far shows that there is a reasonably high divergence in sequence between strains of NDV.

It is hoped that comparisons of the complete viral protein sequences (particularly the glycoproteins HN and F) with those of other strains will help to explain the wide range of virulence observed in isolates of NDV. Comparisons with other members of this viral family (Paramyxoviridae) and with viruses in other families may help to understand the evolutionary relationships between these viruses.

OP10.18

HEMOFUSION-INHIBITION TEST FOR DETECTION OF ANTIBODIES AGAINST NEWCASTLE DISEASE VIRUS. E. Trybała. Department of Veterinary Microbiology, Faculty of Veterinary Medicine, Academy of Agriculture and Technology in Olsztyn, Poland.

Newcastle disease virus (NDV) was shown to cause extensive fusion of erythrocytes at pH 5.5 . Based on this, two versions of the hemofusion-inhibition test (HFI-1 and HFI-2) were established and applied for the detection of antibodies against NDV in chicken sera. Statistical analysis of data revealed a good correlation of the HFI-1 and HFI-2 antibody titers with those obtained in the standard hemagglutination-inhibition (HI), virus neutralization (VN) and hemolysis-inhibition (HLI) tests. HFI-2 antibody titers were lower than HFI-1 ones. This probably resulted from the different procedures employed in these tests. Namely, in the HFI-1 test, similarly as in the HI and HLI tests, virus and serum were mixed prior to the addition of erythrocytes. On the other hand, in the HFI-2 test serum was added to the virus already adsorbed at low temperature to erythrocytes. At this temperature virus particles do not penetrate into the cell. Therefore, there is a theoretical possibility to detect other antibodies than those estimated in the standard HI and HLI tests.

OP11.1

IMMUNOHISTOLOGICAL IDENTIFICATION OF CELLS EXPRESSING K-PAPOVAVIRUS T ANTIGEN IN TISSUES OF ACUTELY INFECTED MICE. John E. Greenlee, M.D. Neurology Service, Veterans Administration Medical Center and Department of Neurology, University of Utah School of Medicine, Salt Lake City, UT, USA.

K virus is a murine papovavirus which produces a fatal interstitial pneumonia in newborn mice. The major target cell for productive K virus infection is the vascular endothelial cell. It is not known, however, whether the virus causes nonproductive infection of other cell types.

Glycol-methacrylate-embedded organs from newborn outbred swiss mice moribund with K virus pneumonia were sectioned at 2 um thickness. Adjacent sections were stained by indirect immunofluorescence methods for K virus T or V antigens. Specific immunofluoresecent labelling of K virus T and V antigens was detected in vascular endothelial cells of multiple organs and also in cells within brain parenchyma. Cell populations expressing T but not V antigen were not detected. These data suggest that K virus infection, as indicated by production of both early and late viral antigens, is confined to vascular endothelial cells and occasional brain parenchymal cells, without involvement of other cell types.

OP11.2

DIMINUTION OF IL-1 PRODUCTION BY CULTURED THYMIC EPITHELIAL CELLS FOLLOWING VIRAL INFECTION. L. De Stephano, H. Goldman, K. Numazaki, I. Wong, M.A. Wainberg. Montreal Children's Hospital Research Institute and Jewish General Hospital, Montreal, Quebec, Canada.

Human thymic epithelial (TE) cells play an important role in T cell maturation and differentiation, and are known to be good producers of interleukin-1 (IL-1) activity. We have studied the effect of viral infection of TE cells, derived from cases of pediatric cardiovascular surgery, and associated thymic lymphocytes, on both production of and responsiveness to IL-1 and IL-2. Infection of TE cells by either cytomegalovirus (CMV) or respiratory syncytial virus (RSV) led to detection of progeny virus after 1-3 weeks in tissue culture, even though no cytopathic effects (CPE) could be observed. Production of IL-1 activity, as measured in a thymic lymphocyte stimulation assay, was completely eliminated by 7 days after infection. Mixture experiments indicated that direct infection of TE cells, rather than production of inhibitory factors, was responsible for this effect. In other studies, infection of thymic lymphocytes by CMV rendered these cells unresponsive to both purified recombinant IL-1 and IL-2. Other viruses, such as influenza, had no effect on production of IL-1-like activity by cultured TE cells. These data suggest that some types of viral infection might be able to compromise the ability of TE cells and thymic lymphocytes to perform immunoregulatory functions.

OP11.3

NEUTRALIZING HUMAN MONOCLONAL ANTIBODIES TO CYTOMEGALOVIRUS. S.Foung*, S.Perkins*, E.Lennette+, P.Bradshaw*, G.Reyes°. Department of Pathology*, Stanford University, Stanford, CA, Virolab+, Berkeley, CA, Genelabs°, Redwood City, CA USA.

Cytomegalovirus (CMV) infection causes significant morbidity and mortality in immunocompromised individuals. The administration of antibodies to CMV may prevent or modify primary infection. We report the in vitro production and initial analysis of human monoclonal antibodies (Mab) to CMV. A human-mouse cell line, SBC-H20, has been developed which forms stable human Ig secreting hybridomas. With this cell line and B lymphocytes isolated from CMV seropositive individuals, multiple hybridomas have been produced secreting IgG human Mabs to CMV. Three Mabs were identified by an enzyme-linked immunoassay and confirmed by indirect immunofluorescence. The pattern of fluorescence with each Mab suggested different CMV-associated epitopes. Mab Z01 immunoprecipitated proteins at 115K, 66K and 50K molecular weight (MW). Mab Z02 immunoprecipitated a major protein of 47-49K MW and lesser proteins at 65K and 130K MW. Mab Z10 identified a protein at 66K MW. By a standard assay, all three human Mabs neutralized CMV at less than 1 µg/ml of specific Ig. All three Mabs are IgG_1 with a kappa light chain. The production of human Mabs to CMV should provide an important new approach to the study of the host response to this agent and form the basis of a potentially unlimited source of relevant human Mabs for treatment of individuals at high risk for life threatening CMV infection.

OP11.4

GASTROINTESTINAL VIRAL INFECTIONS IN SYMPTOMATIC HIV SEROPOSITIVE HOMOSEXUAL MEN. G.Grohmann, J.Harkness, D.Cooper, C.Law and A.L. Cunningham, Virology Department, Westmead Hospital, Sydney, St. Vincent's Hospital and STD Clinic, Sydney Hospital, N.S.W., Australia.

110 stool samples from 72 homosexual men with AIDS or ARC and diarrhoea were examined for gastrointestinal viruses by electron microscopy, isolation in cell culture and Latex agglutination or ELISA (for rotaviruses). 46 stool specimens from 37 men were positive for one or more viruses. Rotavirus was detected in 25 patient's stools. Seven of these had dual infections (5 Adenovirus, 1 CMV, 1 Enterovirus). Another 10 patients had adenovirus in their stools, including two dual infections (CMV and Coronavirus). The other two patients had Echo 11 and Norwalk viruses respectively. 7 of the 15 adenoviruses were detected by electron microscopy alone. In comparison a lower proportion of viruses was detected in the stools of 178 well homosexual men (40% HIV seropositive) who presented concurrently to the Sydney STD Clinic. Rotavirus was detected in 16 patients; adenovirus in 5, coronavirus in two and enteroviruses in 7 patients. There were no dual infections. The high proportion of AIDS/ARC patients with viruses in their stools is similar to results obtained with marrow transplants and suggests a role for immunosuppression in frequency or duration of infection with these viruses.

OP11.5

VIRAL INFECTIONS IN PATIENTS WITH THALASSEMIA MAJOR. G. Nigro, P. Lionetti, G. Multari, C. Di Domenico, S. Maiozzi, S. Ilari, M. Midulla. III Dept. of Pediatrics, "La Sapienza" University and CNR Inst. of Experim. Medicine, Rome, Italy.

A 1-year follow-up on 38 patients (mean age 8.2 yr) with thalassemia major (TM) has been performed by testing complement-fixing antibodies to influenza A and B, parainfluenza 1,2,3, adenovirus, respiratory syncytial virus, rotavirus, cytomegalovirus (CMV), herpes simplex virus (HSV), varicella-zoster and Coxsackie-B-viruses (CBVs). Also, Hepatitis B (HB) markers and antibodies to Epstein-Barr virus (EBV) and Human Immunodeficiency virus (HIV) were tested. As control population, 126 school-children (mean age 8.5 yr) were examined.

By clinical records, only a rotavirus gastroenteritis occurred among TM patients, while the controls had recurrent respiratory diseases associated with viral infections. However, TM patients frequently showed significant antibody movements, predominantly by CBVs, HSV, CMV and EBV; occasionally, specific IgM antibodies by ELISA were also demonstrated.

Antibodies to HIV were found in a 19-year old asymptomatic boy. No HBsAg+ but 5 anti-HBs+ patients were revealed.

Our study showed that TM patients, though immunocompromised, often overcome viral infections having subclinical course.

OP11.6

PRIMARY KILLER-CELL DEFECT AND ACQUIRED ABNORMALITIES OF IgG-1 AND EBV NEUTRALIZING ANTIBODIES IN CHRONIC ACTIVE EPSTEIN-BARR VIRUS (EBV) INFECTION. J.H. Joncas, Y. Monczak, F. Ghibu and C. Alfieri. Departments of Microbiology, Biochemistry and Pediatrics, Ste-Justine Hospital and University of Montreal, Montreal, Quebec, Canada.

We have observed in 3 families a syndrome of chronic active EBV infection characterized by prolonged or recurrent mononucleosis including splenomegaly, anemia and often pancytopenia; it was associated with a primary K-cell defect documented in both EBV seronegative and seropositive members of these families. Various acquired immunological abnormalities, the most constant of which were low NK cytotoxicity, high EBV VCA and EA antibodies and hyper IgG1 were only seen following the EBV infection. The number of circulating EBV genome positive cells was increased. Inconstant features of the syndrome included: lack of ADCC antibodies in 1 patient, ADCC antibodies in low titer in 1 patient, and lack of neutralizing antibodies in at least 1 patient and 2 other family members. The father and daughter in the first family seen with this syndrome show clear evidence of persistent symptoms and immunological abnormalities since 1972 and 1978. The other two families have been followed for a year only. The part, if any, played by the underlying primary K-cell defect documented in these families upon the acquired immunodeficiency remains to be defined.

OP11.7

ASPARTATE-ASSISTED IMMUNE STIMULATION: ITS IMPORTANCE IN ANTITUMOR AND ANTIVIRAL PROTECTION. C. Chany and I. Cerutti. Institut National de la Santé et de la Recherche Médicale, Unité 43, Université René Descartes, Paris V and Département de Physiologie et de Pathologie Virale, Hôpital St. Vincent de Paul, 74 av. Denfert Rochereau, 75674 Paris Cedex, France

Immune stimulators such as Cornyebacterium parvum (CP) are useful for antitumoral and antiviral therapy. However, the immune trigger cannot be reactivated without adversely affecting the disease. We have tried to amplify the results yielded by a single injection of CP by using either interleukin-2 (IL2) or aspartate salts (ASP). In the present report, we show that IL2 has no detectable clinical effect. In contrast, the addition of an ASP salt increases the antiviral and antitumoral protection afforded by the CP-induced trigger. Moreover, treatment using only ASP slightly protects against tumor development and significantly increases antiviral resistance during experimental encephalomyocarditis (EMC) infection. This ASP-assisted CP immune stimulation improves antitumoral resistance even when ascitic tumors have already developed. In the latter case, tumor regression can even be detected. Since ASP increases T-cell cytotoxicity in vitro and aggravates spontaneous T-cell lymphomas in AKR mice, the involvement of T-cell-mediated immunity may explain antitumoral and antiviral effects. We propose the use of this therapeutic model for human cancer therapy, and possibly for treating AIDS.

OP12.1

ENIGMATIC DOUBLE-STRANDED RNA (DSRNA) IN FRENCH BEAN. D.A. Wakarchuk and R.I. Hamilton, Agriculture Canada Research Station, 6660 N.W. Marine Drive, Vancouver, B.C., Canada, V6T 1X2.

Previous studies (Wakarchuk and Hamilton. 1985. Plant Mol. Biol. 5:55-63) showed that dsRNA (c.15 kbp) in French bean cv. Black Turtle Soup (BTS) was, unexpectedly, cellular rather than viral in origin. End-labelled dsRNA fragments and cDNA complementary to the dsRNA hybridized to total DNA, but not to single-stranded RNA, from several plant species. Preliminary mapping using Sma-1, Pst-1 and Xho-1 endonucleases showed that the probes hybridized to c.35% of the sequences in purified BTS chloroplast DNA (c.150 kbp). A recombinant DNA plasmid containing an insert representing 630 bp of the RNA was sequenced. The inserted region is AT-rich and may possibly code for protein. No significant homology between the inserted region and chloroplast DNA sequences was detected by a computer search of sequences in the EMBL 8.0 data base, but the inserted sequence showed 50% homology with a region in the E. coli genome coding for a lambda phage receptor (lamB gene). A smaller region (120 bp) showed 64% homology with a region in the HTLV III retrovirus genome. Neither the biological significance of these homologies nor the function of the dsRNA are known.

OP13.1

BACTERIOPHAGE T7 PROTEIN SYNTHESIS: THE ALKYLATION EFFECT.
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Treatment with methyl methanesulfonate inactivates phages. Alkylation slows down the lytic cycle of bacteriophage T7, as shown by experiments on DNA metabolism and on morphogenesis in Escherichia coli cells infected by alkylated T7 phage. Examination of phage-specific protein synthesis in cells infected by alkylated phage exhibited, once again, the same phenomenon. Using pulse-labelling with [35S]-methionine, we followed the production of the three classes of T7 proteins after infection of irradiated and non-irradiated E. coli cells. Proteins were resolved by electrophoresis on a polyacrylamide gel and detected by autoradiography. Autoradiograms were analysed visually and by densitometry.

Visual inspection revealed a prolongation in the synthesis of class I and class II and class III proteins, as well as a delay in the production of class II and class III proteins. Densitometric analysis showed that the observed delay was, in general, not a delay in the time of first appearance of a given protein but rather a delay in the time of its maximum synthesis. This delay was not simply a delay in the onset of infection, since it was greater for class III proteins than for class II proteins.

Our results support proposed models for the regulation of bacteriophage T7 genetic expression. These models assume: 1) injection of phage DNA linked to its transcription by RNA polymerases and 2) a hierarchy in efficiency of transcription and translation of the different classes of T7 genes.

OP13.2

INTERFERENCE OF VIRAL INFECTION BY DEFECTIVE RNA REPLICASE.
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In order to investigate the function of a conserved segment Tyr-X-Asp-Asp in the RNA-dependent RNA or DNA polymerases, we changed the Gly residue at position 357 in the conserved sequence, Tyr-Gly^{357}-Asp-Asp, of the replicase of RNA coliphage Qβ to Ala, Ser, Pro, Met or Val by site directed mutagenesis and examined the replicase activity in vivo. The cells carrying the variant plasmids lost the replicase activity but prevented severely the proliferation of phage Qβ (group III) and related phage SP (group IV), by supressing the phage RNA synthesis. In contrast, substitution of the Gly residue at position 390 showed a slight inhibitory effect. These results suggest that the cells harboring an altered replicase at conserved segment can interfere specifically with the wild-type phage and different, but related, phage infections. This interfering phenomenon will provide a novel replicase immune system against viral infection.

OP14.1

- PRODUCTION D'ANTICORPS MONOCLONAUX VIS A VIS DU VIRUS RABIQUE : PERSPECTIVES EPIDEMIOLOGIQUES ET INTERET DIAGNOSTIC.

-O.AKACEM (A) BENMANSOUR (M) BRAHIMI ET(M) BENHASSINE

La production d'anticorps monoclonaux (A.C.M) vis à vis d'une souche fixe de virus rabique (C.V.S. 11) nous a permis d'obtenir quatre A.C.M. antiglycoproteine dont trois neutralisants et 11 A.C.M. anti nucléocapside. Une étude épidémiologique de la rage en Algérie par le biais de ces anticorps est actuellement en cours, de même que la comparaison du profil antigénique de souches vaccinales et de souches sauvages isolées chez l'homme et chez l'animal. La standardisation de réactifs de référence à usage diagnostic par le biais des A.C.M anti nucléocapside est également envisagée.

OP14.2

THE APPARENT IMMUNOSUPPRESSION PRODUCED BY HUMAN CYTOMEGALOVIRUS INFECTION OF MONONUCLEAR CELLS IN VITRO MAY BE LARGELY DUE TO MYCOPLASMA CONTAMINATION. Diane Scott and J.G.P. Sissons, MRC Clinical Immunology Research Group, Royal Postgraduate Medical School, London W12 ONS, UK.

Human cytomegalovirus (HCMV) has previously been associated with inducing clinical immunosuppression, and the virus-infected monocyte has been implicated in the production of this effect. Our previous findings suggested that monocytes infected in vitro with HCMV strain AD169 released a potent inhibitor of interleukin 1(IL-1). However, further characterisation of the inhibitory activity showed that our virus stock was contaminated with mycoplasma hyorhinis. We have now found that: (1) mycoplasma free isolates of HCMV - both laboratory passaged strains and recent clinical isolates - failed to cause suppression of mitogen-dependent T cell proliferation, accessory cell function or IL-1 production. (2) The apparent inhibition of proliferation demonstrated by many recent clinical isolates of HC MV obtained from several laboratories correlated directly with the presence of mycoplasma contamination. (3) Isolated mycoplasma hyorhinis inhibited ^{3}H-thymidine uptake in the in vitro assay for T cell proliferation. These findings (together with very limited evidence for HCMV gene expression in mononuclear cells) suggest any in vitro immunosuppressive effects previously attributed to HCMV are unlikely to be due to direct infection of monocytes.

OP15.1

INTERLEUKIN-2 AND EXPERIMENTAL RABIES.

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Interleukin-2 (IL-2) is a lymphokin produced by lymphoid cells (particularly T-helper lymphocytes). Its synthesis occurs after stimulation by an antigen. IL-2 has a variety of immunoregulatory functions. It enhances generation of T-cells, growth of killer T-cells and natural killer cells. Moreover, IL-2 plays an important role in B cell generation.

For rabies it is well known that humoral and cellular immune responses obtained after vaccination are responsible for the virus clearance. Inactivated rabies virus and immunosomes (glycoprotein coated liposomes) induce IL-2 synthesis, but the role of IL-2 in rabies prophylaxis is not clear.

In this study we report that: 1) in animals (Balb/c mice) infected with a lethal dose of wild rabies virus, IL-2 synthesis is inhibited; 2) recombinant human IL-2 is capable protecting some animals (syrian hamsters) against a lethal dose of wild rabies virus; 3) recombinant human IL-2 enhances the protective activity of rabies antigens when used in mice according to a pre-exposure potency test (NIH test).

Other experiments are in progress to clarify the role of IL-2 and to see whether recombinant human IL-2 can be associated with antirabies treatment such as neutralizing antibodies or interferon.

OP15.2

CHARACTERIZATION OF HELPER T CELL CLONES RECOGNIZING INFLUENZA VIRUS HAEMAGGLUTININ.

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Helper T cell clones specific for the haemagglutinin (HA) molecule of influenza virus were isolated from spleen cells of BALB/c and (BALB/c x B10)F1 mice immunized with H3 subtype virus, and lymph node cells from BALB/c mice primed with purified HA. The specificity of these T cell clones was assessed in proliferation assays by 1) reactivity with different strains of virus that have arisen by antigenic drift and have known amino acid sequence changes in HA and 2) reactivity with monoclonal antibody-selected variants which have single amino acid substitutions in the HA. Those variants with mutations that abrogate interaction with a T cell clone were purified and the HA gene sequenced. Sixteen clones were divided into five groups each having a distinct reactivity pattern with the drift strains. Despite these different patterns, the epitopes recognized by four of the groups were found, by reactivity with variant viruses, to be in very close proximity to one another and may even be overlapping. These epitopes are centred around the region of amino acid residues 60-63 of the HA molecule designated, from antibody binding studies, as Site E.

OP15.3

ELEMENTS OF ENHANCED PERIPHERAL BLOOD MONONUCLEAR CELL (PBMC) MEDIATED LYSIS OF CMV TARGETS DURING CMV INFECTION.

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Fresh PBMC and fractionated subpopulations were obtained from adult strains 2, 13, and OM3 guinea pigs one month after inoculation with guinea pig CMV and assayed in parallel with effectors from uninfected animals. Lysis of MHC-matched or mismatched CMV targets in ^{51}Cr release assays was comparable among inbred strains. Lysis of CMV targets was enhanced in PBMC from CMV-infected compared to uninfected animals ($p<.01$). Lysis of HSV targets was unaffected by CMV infection. Lytic activity against CMV targets resided in high buoyancy, Percoll-separated, LGL-containing fractions in uninfected animals but extended into low buoyancy cells in CMV-infected animals. CMV target lysis in nonadherent PBMC was 2/3 that in whole PBMC from infected and uninfected animals. Adherent PBMC from infected animals had greater lytic activity (34%) than those from uninfected animals (<8%). Enhanced lysis by whole PBMC was associated with in vitro production of interferon and enhancement was abrogated by depletion of adherent plus SIg bearing cells. Thus enhanced in vitro CMV-specific PBMC mediated cytotoxicity during in vivo infection is not MHC-restricted, has contributions from several cells types, and is associated with in vitro production of interferon.

OP15.4

CELLS EXPRESSING HERPES SIMPLEX VIRUS GLYCOPROTEIN gC BUT NOT gB, gD or gE ARE RECOGNIZED BY MURINE VIRUS-SPECIFIC CYTOTOXIC T LYMPHOCYTES.

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In order to determine which viral molecules(s) specify recognition by herpes simplex virus (HSV)-specific cytotoxic T lymphocytes (CTL), the technique of DNA-mediated gene transfer was used to construct syngeneic target cells which express individual HSV glycoprotein molecules. Previously, a mouse L cell line, I4/6, which constitutively expressed very high levels of HSV-2 gD was isolated. Despite the expression of gD-2 on the surface of I4/6 cells, these cells were not killed by anti-HSV-2 CTL generated following intravaginal infection of syngeneic mice. In contrast, parental I4 or I4/6 cells infected with HSV-2 were lysed. Additionally, cells which express HSV-1 gB, gC or gE were isolated following appropriate transfection and selection. The relative level of glycoprotein expression was examined in each case. Anti-HSV-1 CTL generated following footpad infection of syngeneic mice were unable to lyse target cells expressing gB-1 or gE-1. In contrast, target cells expressing barely detectable levels of gC-1 were killed by syngeneic anti-HSV-1 CTL as well as HSV-1 infected target cells. These results suggest that anti-HSV CTL do not recognize gB-1, gD-2 or gE-1 as expressed in transfected target cells, but do recognize gC-1 as a target.

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OP15.5

INDUCTION OF CLASS I AND CLASS II TRANSPLANTATION ANTIGENS IN RAT BRAIN DURING FATAL AND NON-FATAL MEASLES VIRUS INFECTION.

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[1]Department of Pathology and [2]Neurology, Huddinge University Hospital, [3]Department of Virology, SBL, Karolinska Institute, Stockholm and [4]Department of Medical and Physiological Chemistry, Uppsala University, Uppsala. Sweden.

Measles virus induced a marked increase in the expression of MHC-coded class I and class II antigens as detected by immunostaining during both fatal and non-fatal brain infections in rats. The distribution of these molecules in the brain was much more widespread than the occurrence of viral antigen suggesting a soluable factor for their induction. In 14-day-old rats with non-fatal infection there was a marked infiltration of T lymphocytes of "cytotoxic/suppressor" phenotype in the brain parenchyma, whereas T "helper" cell phenotypes mainly were located perivascularly. In brains from newborn rats with a fatal infection no or only few lymphocytes were detected.

OP15.6

RELATIVE CONTRIBUTION OF POLYMORPHONUCLEAR LEUKOCYTES AND OF ALVEOLAR MACROPHAGES TO PROTECTION DURING THE EARLY PHASE OF INTRANASAL INFECTION OF INFLUENZA VIRUS.

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*Department of Immunology, Medical Institute for Bioregulation Research, Kyushu University, Fukuoka, Japan.

The relative contribution of polymorphonuclear leukocytes (PMN) and macrophages in the early protection against intranasal infection of mice with influenza virus was investigated. Virus propagation in the lung in the early phase of infection with less than 1.5×10^3 plaque forming units (PFU) was enhanced by X-irradiation but not by intranasal administration of carrageenan. When mice were infected with 1.5×10^4 PFU, the virus titre was elevated by X-irradiation as well as the intranasal administration of carrageenan. The intranasal administration of carrageenan not only inhibited the phagocytic activity of alveolar macrophages but not enhanced susceptibility to the virus. PMN were capable of phagocytosing the virus and were non-permissive for virus infection. Neutralizing antibody and interferon were not detectable in the early stage of the infection. These results suggested that PMN (X-ray-sensitive, carrageenan-resistant) were the cells primarily responsible for early protection in influenza virus infection and that after infection with a high dose of the virus alveolar macrophages (X-ray-resistant, carrageenan-sensitive) also played a protective role in the early phase.

OP15.7

INHIBITION OF CMV REPLICATION IN HUMAN FIBROBLASTS BY HUMAN MONOCYTES AND MONOCYTE-DERIVED MACROPHAGES

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The influence of monocytes (M) and monocyte-derived macrophages (MdM) on the fate of cytomegalovirus (CMV) in human fibroblasts (HF) was studied by one step growth experiments, dot hybridization and electron microscopy. M or MdM were added at a ratio of 2:1 to CMV infected HF after virus adsorption. Infectious virus yield reductions of 5 and 100 fold by M and MdM respectively were observed with HF infected at an moi of 1. The presence of specific antibodies caused additional reductions of about 10 and 4 fold (M and MdM respectively). Dot hybridization experiments with a ^{32}p labeled probe showed an inhibition of viral DNA production. Reductions of 50% and 90% of cpm bound resulted from the presence of M and MdM respectively. Electron microscopy showed significant ribosomal activity in the cytoplasm of the CMV infected HF, while the nuclei contained aggregates of viral capsids. In the CMV infected HF treated with M or MdM, relatively few CMV capsids were seen in the nuclei. Most of these cells contained many vacuoles and enlarged mitochondria. The significance of these findings in primary and recurrent CMV infections in vivo will be discussed. This work was supported by the Middle East Eye Research Institute. (MEERI)

OP15.8

HAEMAGGLUTINATION INHIBITION (HAI) ANTIBODY LEVELS ONE YEAR AFTER NATURAL MEASLES INFECTION AND VACCINATION IN NIGERIAN CHILDREN

BY N.O. Eghafona, L.E. Odama, S.O. Emejuaiwe, A.A. Ahmad and C.D. Ezeokoli. Department of Microbiology, Ahmadu Bello University, Zaria, Nigeria.

An assessment of haemagglutination inhibition antibody titres in 1163 children, comprising of 739 recipients of live measles vaccines and 424 patients with natural measles infection one year after was made in this investigation. Statistical analysis by the Mann-Whitney test at $x = 0.05$ of the data obtained revealed a significant difference in the levels of HAI antibodies. A significant 67.45% of vaccinated children showed antibody titres of $\leq$ 1:16, while 23.48% of children with natural measles showed such titres.

The importance/implication of HAI antibody titres of $\leq$ 1:16 is discussed.

OP15.9

FUNCTIONAL ANALYSIS OF HUMAN T CELLS SPECIFIC FOR HEPATITIS B AND RABIES VIRUSES.

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T cell lines specific for hepatitis B surface antigen (HBsAg) or rabies antigens were isolated from human vaccine recipients by stimulating their peripheral blood mononuclear cells in culture with viral antigens in the presence of interleukin-2 (IL-2). Long-term culture T cell lines were shown to proliferate specifically in the absence of IL-2 to the viral antigens (HBsAg or inactivated rabies virus). In addition, the rabies-specific T cells could be induced to proliferate by most of the viral components such as the glycoprotein (G), nucleoprotein (N), and the matrix (M) and non-structural (NS) proteins. At the same time, the viral-specific T cell lines displayed strong cytotoxicity against target cells expressing surface viral antigens and DR molecules. B cell lymphoblastoid cells that were incubated overnight with HBsAg or synthetic peptides of HBsAg or infected with a vaccinia-HBsAg recombinant virus were killed with high efficiency by the HBsAg-specific T cells but not by the rabies-reactive T cells. Similarly, the rabies-specific T cells displayed cytotoxic activity towards lymphoblastoid cells that were infected with a vaccinia-rabies G recombinant virus or alternatively incubated overnight with inactivated rabies virus, purified ribonucleoprotein, NS protein.

OP15.10

VACCINIA RECOMBINANT VIRUSES EXPRESSING HUMAN CYTOMEGALOVIRUS MAJOR IMMEDIATE EARLY PROTEIN AND GLYCOPROTEIN B ACTIVATE AND ARE RECOGNISED BY HCMV SPECIFIC CYTOTOXIC T CELLS.

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Human cytomegalovirus (HCMV) specific cytotoxic T (Tc) cells recognise both structural (LA) and non-structural (IEA -EA) antigens in infected cells. To delineate which proteins are recognised, recombinant vaccinia viruses encoding the structural glycoprotein B (vac.gB) and the major 72kDa IEA (vac.mIEA) were constructed. These antigens were expressed in infected human fibroblasts and such cells were co-cultured with autologous T cells with IL2 for 14d. Vac.gB stimulated T cell lines killed gB but not IEA expressing cells and such T cell lines killed autologous 72h HCMV infected cells. To determine the relative numbers of precursor Tc against these two viral proteins, limiting dilution cultures were established with vac.gB, vac.mIEA and HCMV infected autologous fibroblasts. Clonal Tc stimulated with recombinant virus killed HCMV infected cells, but HCMV stimulated Tc clones killed mainly vac.mIEA infected cells, although a few recognised vac.gB infected cells. This extends our observations concerning stage specificity of HCMV Tc and suggests that the 72kDa IEA is an immunodominant antigen. A much smaller number of HCMV specific Tc recognise LA such as glycoprotein B.

OP15.11

RECOGNITION OF HEAT-INACTIVATED INFLUENZA VIRUS BY CLASS I AND CLASS II RESTRICTED VIRUS-SPECIFIC MURINE CTL CLONES

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Currently used subunit or inactivated influenza vaccines generally induced poor or negligible cytotoxic T lymphocyte (CTL) immunity. Since evidences that anti-influenza virus CTL play an important role in defence against virus infection have been accumulated, influenza virus vaccines to elicit powerful CTL immunity is recommended.

We found that influenza virus heat-inactivated at 55C for 30 min retained fusion activity and the capabilities to elicit CTL and to sensitize target cells for lysis by CTL. In this study, we report on differences in patterns of recognition of targets and of protection of mice with class I and II restricted CTL clones. Class I restricted clones recognized target cells sensitized by a wide range of dose of this heat virus, whereas class II ones did target cells sensitized by a narrow range of this heat virus or other HA containing antigens. The former recognized nucleoprotein (NP) antigen and other inner one of the heat virus but not its HA antigen, whereas the latter did HA antigens. Cross-reactive class I clones protected mice from lethal virus infection, while class II clones did not, but rather tended to shorten the period to death. Vaccination of mice with heat-inactivated virus protected mice from lethal infection by challenge virus.

OP15.12

PROTECTION AGAINST PHAGE INFECTION USING ANTISENSE RNAs OF RNA COLIPHAGE GENOME.

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An antisense RNA to a specific mRNA has been shown to function as a repressor for gene expression in E. coli. This finding led to the development of strategies to artificially regulate gene expression and create a novel immune system against viral infection.

Recently, we have developed a specific E. coli immune system against phage infection. The E. coli cells containing a plasmid carrying an inducible gene for antisense RNAs against viral genes became resistant or immune to the phage upon induction of the antisense RNA. In the RNA coliphage SP , the gene for the maturation protein was found to be the best target for this type of immune system. Moreover, the strong inhibitory effect was observed with only a 19-base sequence covering the Shine-Dargarno sequence without the coding region of the maturation protein. Using various deletion mutants in the 5'-terminal untranslated region, we will discuss minimum size of the antisense RNAs against SP infection in the presentation.

OP15.13

ADOPTIVE TRANSFER BY DENDRITIC CELLS IN MOUSE HERPETIC INFECTION.

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Dendritic Cells (DC) possess the capability to present viral antigens on their surface. In previous experiments we described murine lymphonode DC obtained from Herpes Simplex Virus (HSV) sensitized mice able to induce proliferation of a HSV-specific helper T-cell clone. These findings stressed us to investigate the possibility to protect BALB/c and C57BL/10 mice from HSV-2 challenge by lymphonode DC obtained from HSV-2 sensitized syngeneic donors (DCs).The following aspects were analysed, namely: the mortality rates, the virus yield in the liver, the specific immune response.

The results obtained demonstrate a powerfull capability of DCs to protect mice from HSV-2 infection as well as to elicitate a specific immune response versus HSV-2.

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OP15.14

PREPARATION OF HUMAN-MOUSE CYBRIDS THAT RESPOND TO HUMAN INTERFERON-γ AND ACQUIRE ANTIVIRAL STATE.

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According to pleiotypic effets of IFN-γ, it is suspected that several type of receptors are existed on various cells of different functions. Here, for serving analysis of molecular and functional heterogeneity of receptors for human IFN-γ, we prepare human-mouse cybrids that respond to human IFN-γ and become antiviral state.

Human amnion-derived WISH cells and mouse L cells were fused with Polyethyleneglycol 4,000. Cells that responded to human IFN-γ and acquired remistance againsyt cytopathic effect of Vesicular stomatitis virus(VSV) were isolated by using anti-VSV rabbit serum and cloned.

By this method, 9 clones were selected and reffered to as HLW-1 to HLW-9. All 9 clones had exactly fused karyotypes of human and mouse cells that were indicated by H33258 staining. All 9clones have responsiveness against challenge with human IFN-γ much more intense than WISH cells but lost responsiveness against murine IFN-α/β between several passages. Intensity of response against human IFN-γ indicated little difference between clone and clone. Ratio of response intensity against human IFN-γ was 4 to 10 in the case of HLW-3 and HLW-5. These cybrids presumably be able to serve the analysis of function of receptor(s) against human IFN-γ .

OP16.1

THE DEVELOPMENT OF MVM, AN AUTONOMOUS PARVOVIRUS, AS A POTENTIAL EUKARYOTIC CLONING VECTOR. R.O. Shade and C.R. Astell. Department of Biochemistry, Faculty of Medicine, University of British Columbia, Vancouver, British Columbia, Canada.

A clone of the Minute Virus of Mice (MVM) genome has been available as a double-stranded insert in pBR322 for many years. This clone, pMM984 [Merchlinsky, M.J., et al., (1983), J. Virol. 47, 227], is being used as a basis for the development of an autonomous parvovirus eukaryotic vector to be used for either transient or prolonged expression of eukaryotic DNA.

Initial studies measured production of infective virus after transfection of uncut pMM984 and of pMM984 cut with various enzymes that either linearize the plasmid or release the viral insert. Later small mutations were introduced and their effect on viral replication and virion maturation was studied. A dominant selectable marker was also introduced at various points in the viral genome. The ability of these constructs to replicate, mature into viruses and/or confer drug resistance was noted. All constructs were examined as to the stability of the drug resistance (if applicable) and as to the nature of the transformed DNA.

Supported by MRC (Canada) and BCHCRF.

OP16.2

NOVEL PSEUDORABIES VIRUS (PRV) MUTANTS EXPRESSING GLYCOSYLATED FUSION PROTEINS OF PRV gIII AND HIV ENV. M. E. Whealy, K. Baumeister, A. K. Robbins, L. Enquist, E.I. DuPont de Nemours & Co.,Inc.,Wilmington,DE 19898 USA

We have constructed three PRV mutants in which the non-essential gene coding the envelope glycoprotein gIII is replaced with gIII/HIV hybrid genes. The hybrid genes were constructed in an E. coli expression vector and subsequently transferred to the Becker strain of PRV by co-transfection of plasmid DNA and viral DNA in PK15 cells. Mutant viruses were selected by their inability to react with a gIII specific monoclonal antibody in the black plaque assay. The mutant viruses were designated PRV-24, PRV-33 and PRV-37.

All of the hybrid genes contain the transcription control sequences and the first 468 bp of the PRV gIII gene fused to different envelope regions of the human retrovirus HIV. PRV-24 contains the HIV envelope portion encoding the conserved C-terminal portion of gp 120 and all the predicted gp 41 sequences. PRV-33 contains this same portion of gp 120, but only 70% of gp 41. The final mutant, PRV-37 contains only the sequences encoding gp 120. All of the mutant viruses express novel fusion proteins which are immunoprecipitated by polyvalent gIII specific sera as well as AID's patient sera. Further work characterizing the properties of these novel fusion proteins will be presented.

OP16.3

INTRODUCTION OF RECOMBINANT DNA INTO A PSEUDORABIES VIRUS VECTOR BY SITE SPECIFIC RECOMBINATION. B. Sauer, M. Whealy, A. Robbins, L. Enquist, E. I. DuPont de Nemours & Co., Inc. Experimental Station, E328,Wilmington, DE 19898 USA

A simple efficient method for introducing recombinant DNA into pseudorabies virus (PRV) has been developed. Recombinants are formed using the Cre-lox system of coliphage P1 in which a single protein, the Cre recombinase, can cause intermolecular site specific recombination between two DNA molecules, if each contains a 34 bp lox site, to generate a recombinant molecule. Incubation in vitro of a circular plasmid DNA containing a lox site with viral DNA from PRV22, a PRV vector which contains a single lox site, in the presence of the Cre protein results in the generation of 2-5% recombinant molecules. These recombinants contain the circular plasmid integrated into the PRV DNA at the lox site. Transfection of PK15 pig kidney cells with the reaction mix yields 2-5% recombinant virus.

Recombinant virus are readily identified by a black plaque assay using a monoclonal antibody against the PRV gIII glycoprotein. PRV42 contains a lox site in the gIII gene and produces a fusion protein still recognized by the gIII monoclonal antibody. Hence, PRV42 forms a black plaque. Integration of a circular plasmid at the lox site disrupts the gIII gene, abolishing the epitope recognized by the antibody. The resulting viral plaques are thus white and easily detected.

The procedure is generally applicable to other viruses.

OP16.4

EXPRESSION OF BOVINE ROTAVIRUS NEUTRALIZATION ANTIGEN IN E. coli. M. Francavilla, V. Gagliardi, P. Miranda, A. Di Matteo, A. Sarasini, G. Gerna, and G. Milanesi.
Ist. Genetica Biochimica ed Evoluzionistica CNR, and Virus Lab, Inst. Infectious Diseases, University of Pavia, 27100 Pavia, Italy.

A 646 bp fragment, encoding 216 of the 326 aminoacids constituting VP7, the major neutralization (Nt) antigen, was derived from a full length cDNA clone of genomic segment 9 of bovine rotavirus (NCDV strain), and inserted into E. coli expression plasmid pEX1. Several transformed bacterial clones were isolated in which the recombinant plasmid directed the synthesis of a cro-β-galattosidase -VP7 fusion protein. Sera from rabbits immunized with the fusion protein specifically stained VP7 among NCDV virion peptides in Western blotting tests. The chimeric polypeptide was also specifically recognized by three Nt monoclonal antibodies to NCDV VP7. Partially supported by CNR Prog. Finalizzato Controllo Mal. da Infezione and Min. Sanità, IRCCS Pol. S.Matteo, Ric. corrente 1985.

OP16.5

USE OF REGULATORY ELEMENTS DERIVED FROM ONE OF THE MOST STRONGLY-EXPRESSED GENES OF COWPOX VIRUS, TO GAIN HIGH LEVELS OF EXPRESSION OF GENES INSERTED INTO POXVIRUS GENOMES. D. Patel, C. A. Ray, and D. J. Pickup. Department of Microbiology and Immunology, Duke University Medical Center, Durham, NC 27710 USA

One of the most abundant viral proteins in a poxvirus-infected cell is the protein that is the major component of the large, poxvirus-induced, A-type inclusions (ATIs). Coupox virus (CPV) produces ATIs whose major component is a 160 kDa protein. During late stages of infection this protein may constitute up to 4% of the total protein of human 143 cells.

We have constructed a plasmid vector which can be used to insert genes into a poxvirus genome such that the expression of the inserted gene is directed by elements derived from those controlling the expression of the CPV gene encoding the 160 kDa protein. High levels of expression of the bacterial chloramphenicol acetyltransferase gene have already been achieved with this system. In order to optimize the system we are investigating the effects of altering: (1) the nature of the regulatory elements; (2) the positions of the regulatory elements relative to the coding region of the inserted gene; and (3) the position and orientation of the hybrid gene within the viral genome.

OP16.6

EXPRESSION OF TOBACCO MOSAIC VIRUS RNA IN TRANSGENIC PLANTS J.Yamaya, M. Yoshioka, *T. Meshi, *Y. Okada, **T. Ohno. Plant Bioengineering Lab., Kirin Brewery, Kitsuregawa-machi, Tochigi, *Department of Biophysics and Biochemistry, Tokyo University, Tokyo, **Department of Agricultural Chemistry, Tokyo University of Agriculture and Technology, Fuchu, JAPAN.

Tobacco mosaic virus (TMV) is a message-sense, single-stranded plant RNA virus with a nondivided genome of 6.4 kb in length. We and another group have reported the synthesis of infectious viral RNAs by in vitro transcription from a cDNA copy of the TMV RNA. Here we describe the introduction of the cDNA copy of the TMV RNA into the genomic DNA of tobacco plants by the Ti plasmid mediated transformation, and the expression of infectious viral particles in planta.

A full-length cDNA copy of the genomic RNA of a TMV strain called TMV-L was put downstream of the cauliflower mosaic virus 35S promoter which had been modified so that the transcription would start just from the 5' end of the viral cDNA sequence. Five tobacco plants (cv. Samsun) transformed with this promoter-cDNA construct were obtained and all were proved to be transgenic by the Southern blot analysis. Every leaf of the five transgenic tobaccos showed typical symptoms of the TMV infection, i.e., mosaic and wrinkling. Leaf homogenates of the transgenic tobaccos were infectious upon a local lesion assay, which indicates that the symptoms in the transgenic tobaccos are due to the multiplication of infectious TMV particles.

OP16.7

THE MOLECULAR CLONING OF COMPLEMENTARY DNA COPIES OF THE BIPARTITE GENOME OF SWEET CLOVER NECROTIC MOSAIC VIRUS. R.D. Gietz[1], D.K. Lakshman[2], C. Hiruki[2], and J.M. Tyler[1]. [1]Department of Genetics, [2]Department of Plant Science, University of Alberta, Edmonton, Alberta T6G 2P5, Canada.

Complementary DNA (cDNA) copies of sweet clover necrotic mosaic virus (SCNMV) genomic RNAs have been cloned into the E. coli vector pUC19. Isolated virion RNA was polyadenylated and then used to produce cDNA with AMV reverse transcriptase after priming with oligo dT. E. coli DNA polymerase 1 and RNase H were then used to produce double stranded cDNA, which was then methylated and blunt end ligated to synthetic Eco R1 linkers. Size fractionated Eco R1 digested cDNA was then ligated into Eco R1 digested pUC19.

Plasmids with inserts homologous to RNA 1 and RNA 2 of SCNMV have been identified using northern gel blot hybridization. Two clones (pDL10, pDL2), with insert sizes of 1.75kb and a 1.4kb, have been shown to be complementary to RNA 1 and RNA 2 respectively. Significant homologies between SCNMV and other members of the dianthovirus group, namely, carnation ring spot virus and red clover necrotic mosaic virus were noticed in a northern gel blot hybridization probed with the SCNMV clones. Work is in progress to characterize the SCNMV subgenomic RNAs of infected plants.

OP16.8

CONSTRUCTION OF AVIAN VACCINE VECTORS FROM FOWLPOX VIRUS: MAPPING AND CHARACTERIZATION OF THE FPV THYMIDINE KINASE GENE. Christopher Beisel and Keyvan Nazerian. U.S. Dept. of Agriculture, Agricultural Research Service, Regional Poultry Research Laboratory, East Lansing, MI USA.

We are mapping and characterizing genes of fowlpox virus (FPV) in order to identify regions of the viral genome where foreign DNA may be inserted and expressed. The similarity of the DNA sequences of thymidine kinase (tk) genes from a variety of organisms suggested the possibility of using a vaccinia virus tk probe to detect the FPV tk gene by blot hybridization.

We developed a low-stringency hybridization protocol that allowed us to map the FPV tk gene to a 1.2 kb AccI fragment within the 5.6 kb FPV EcoRI Q fragment. The DNA sequence of this fragment reveals an open reading frame with short regions of homology to the vaccinia tk gene, including a 21 bp region near the 5' end that others have hypothesized to code for the tk nucleotide binding site. Sites for initiation and termination of transcription were mapped by S1 nuclease analysis. A comparison of the 5' and 3' non-coding regions with those reported for vaccinia virus early genes will be presented. We will also report on attempts to use this technique to map other FPV genes for which corresponding vaccinia probes are available.

OP16.9

EXPRESSION AND SECRETION OF HUMAN INTERFERON GAMMA IN FILAMENTOUS FUNGUS. Wai-Choi Leung & Maria Leung. Depts. of Medicine, Medical Microbiology & Infectious Diseases, University of Alberta, Edmonton, Alberta T6G 2G3

Filamentous fungi can glycosylate and secrete proteins. It is a valuable host cell for expression of recombinant proteins requiring protein processing. Previously, we have expressed the herpes simplex virus thymidine kinase for aquatic filamentous fungus Achlya ambisexualis. Fermentation studies were able to increase the biomass yield by 8 fold in fermentor. We then proceeded to express human interferon gamma (HuIFN-γ) in order to assess the ability of the fungal cell for glycosylation and secretion. We have chemically synthesized the structural gene for HuIFN-γ and also the natural signal peptide. The gene was ligated under the SV40 promoter and used to transform the fungal protoplast. Southern blot analysis of transformed cell DNA suggested the integration of the transforming genomes into cell DNA. RNA blot analysis indicated the presence of HuIFN-γ RNA. The expressed HuIFN-γ was processed into functional protein. From 42% to 46% of the total expressed HuIFN-γ was found in the media. The rest of the HuIFN-γ antiviral activity was intracellular. Other intracellular proteins were not found in the media, excluding the leakage of proteins into the media. The secreted HuIFN-γ was glycosylated as demonstrated by incorporation of ^{3}H-glucosamine into the protein molecule. This study indicates that the fungal cell can glycosylate, secrete and process HuIFN-γ protein molecules.

OP16.10

CHARACTERIZATION OF RECOMBINANT HERPES SIMPLEX VIRUS THYMIDINE KINASE EXPRESSED IN FILAMENTOUS FUNGUS. Wai-Choi Leung, Depts. of Medicine, Med. Micro. & Infectious Diseases, University of Alberta, Edmonton, Alberta, Canada T6G 2G3.

We have developed the filamentous aquatic fungus Achlya ambisexualis as a host cell for expression of foreign proteins. Herpes simplex virus thymidine kinase (HSV TK) was used as a marker for assessment. The HSV TK was expressed from a recombinant plasmid used to transform the fungal cell. The transformed cell expressed the HSV TK in the cytosol fraction. A cytosol fraction of LTK^{-} cells infected with HSV was used as control viral enzyme preparation. The fungal expressed TK bears similar electrophoretic mobility, isoelectric point and sedimentation coefficient, to those of the viral TK, suggesting similar size and charge. The fungal expressed TK also utilized CTP, GTP, UTP in addition to ATP as phosphate donor; dC, in addition to dT as phosphate acceptor. The enzyme was also inhibited by dTTP, but not dCTP. The fungal expressed TK can also be neutralized by anti HSV-1 antisera, and bears similar heat inactivation kinetics with that of the viral TK. These findings indicate that the fungal cell can express and correctly assemble the TK polypeptide into functional dimer form. On glycerol gradient sedimentation studies, the fungal expressed TK exhibited a major peak, in addition to a slight shoulder with slower sedimentation rate. The shoulder can be prevented by the addition of protease inhibitor, suggesting that proteolytic degradation did occur to the fungal expressed TK.

OP16.11

STUDY OF A LOCAL ISOLATE OF NUCLEAR POLYHEDROSIS VIRUS OF BOMBYX MORI AND THE CONSTRUCTION OF EXPRESSION VECTOR BASED ON ITS POLYHEDRIN GENE. Nien-Tai Hu, Shih Win Ma and Roger F. Hou. Agricultural Biotechnology Laboratories, National Chung Hsing University, Taichung, Taiwan

Deoxyribonucleic acid purified from the polyhedrosis virus isolated in Taiwan was compared with that of a Japanese isolate (from Kobayashi of Nagoya University) for restriction fragment length polymorphism. Distinct differences were observed when DNAs were digested with restriction enzyme Eco RI or Hind III.

A genomic bank of the viral DNA was constructed in λEMBL3. Two hundred and eighteen recombinant phages were obtained, from which four were found to contain polyhedrin gene based on their hybridization to a polyhedrin gene probe (from Maeda of Tottori University). One such clone, λNPV1, was studied further and used in the construction of expression vector. The polyhedrin gene was located on a 3.9Kb Hind III fragment. Both its location and the restriction map, examined so far, of the 3.9Kb Hind III fragment were identical to that of Maeda's clone, suggesting that the sequences surrounding polyhedrin gene were well conserved. A unique Xba I site downstream of the polyhedrin gene promoter was utilized in the construction of expression vector. Sequences between the Xba I site and the promoter were trimmed with Bal-31 and a Bam HI site was inserted for future cloning purpose. The Xba I site was recreated downstream of the Bam HI site during construction.

OP17.1

LARGE-SCALE PURIFICATION AND IMMUNE-ELECTRON MICROSCOPIC CHARACTERIZATION OF BOVINE VIRAL DIARRHEA VIRUS. K.S. Tsai, and J.B. Hopkins, Connaught Laboratories Limited, Willowdale, Ontario, Canada.

In the past few years, attempts to characterize bovine viral diarrhea virus (BVDV), the pathogen of bovine viral diarrhea-mucosal disease complex, have produced varied results regarding its physical and morphological characteristics. To our view, the lack of sufficient quantity of well purified virus preparation contributed an important factor to this descrepancy. We have concentrated and purified BVDV from large volumes of virus harvests by continuous-flow zonal centrifugation (CFZC) using sucrose as gradient. After 2nd CFZC, the density of BVDV was determined to be between 1.110 and 1.150 gm/cm^3 with maximum infectivity at 1.132 gm/cm^3.

Aliquots of purified virus preparations, taken from 3 peak fractions of 2nd CFZC, were examined by immune electron microscopy (IEM). Aggregates of virus particles-antibody interaction were observed in all 3 fractions, but the fraction with sucrose density at 20% (equivalent to 1.132 gm/cm^3) showed more homogeneous sized particles. These virus particles consisted of a membranous coat and a central cord and measurements of diameters ranged from 52 to 68 nm with a mean of 58 nm.

OP17.2

MENGO VIRUS MATURATION IS ACCOMPANIED BY C-TERMINAL MODIFICATION OF CAPSID PROTEIN VP1. Ulrike Boege and Doug Scraba. Dept. of Biochem., Univ. of Alberta, Edmonton, T6G 2H7 Canada.

In the three dimensional structure of Mengo the C-terminal end of VP1 is located at the bottom of the "pit", and the last amino acid detectable in the electron density map is proline 259 (Luo *et al.* Science 234: 182, 1987). The chemically determined C-terminus of VP1 in purified virions is leucine 274 (Ziola and Scraba, Virology 71: 111, 1976). In contrast, the C-terminus of VP1 synthesized in reticulocyte lysates is glutamate 277 (A. Palmenberg, pers. comm.). It is therefore possible that the chymotrypsin treatment used during virus purification produces the cleavage at leucine. We have confirmed the C-terminal leucine in virions purified in the usual manner, and have found that virions purified without using chymotrypsin contained a mixture of VP1 molecules: ~2/3 ending with leucine and ~1/3 ending with glutamate. Since at the time infected cells were harvested for purification about 2/3 of the virions had been released into the supernatant, the results of the C-terminal analyses suggest that a final "trimming" of three residues from VP1 is part of the maturation process. Presumably this is accomplished by a cellular enzyme with chymotrypsin-like activity. The virions purified without chymotrypsin include a population resistant to dissociation in the presence of Cl^- ions at pH 6. This may indicate that the "trimming" of VP1 is necessary for the efficient uncoating of virions in the next infectious cycle. We are currently assaying the relative infectivity of non-chymotrypsin treated virions.

OP17.3

IMMUNOGENICITY OF PURIFIED FLAVIVIRUS GLYCOPROTEINS

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Recent investigations have shown that immunization with the non-structural glycoprotein, NS1, of yellow fever virus results in protection of mice on subsequent challenge with live virus. In addition, mice were also found to be protected by passive immunization with anti-NS1 monoclonal antibodies. Since antibody mediated enhancement of virus replication in macrophages has been implicated in the pathogenesis of dengue haemorrhagic fever, the significance of these findings for the development of vaccines against the dengue virus group have been widely discussed in terms of an induction of immunity without stimulating antibodies to virion surface components.

In this study we investigated the immunogenicity of the NS1 glycoprotein of dengue type 2 virus (PR159). The glycoprotein was purified on a monoclonal antibody affinity column from solubilized extracts of infected cells grown on microcarrier beads. Both monomer and micellar forms were prepared, adsorbed to alum and assessed for immunogenicity in mice. The results of the dose-dependent response to these sub-unit preparations will be discussed.

OP17.4

FURTHER CHARACTERIZATION OF RUBELLA VARIANT HPV-RV.

M.Urade, T.Mima, T.Matsuya, and *K.Shiraki, 1st Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, and *Research Institute for Microbial Diseases, Osaka University, Suita, Osaka, Japan.

Rubella variant HPV-RV, isolated from high passaged rubella virus (RV) stocks grown on BHK21, carries CF antigen of RV, but its infectivity and hemagglutinating activity were inhibited by antiserum against a latent (R-type) virus of BHK21 but not by anti-RV serum. It also carries DNA polymerase activity derived from a latent virus (Arch Virol 56: 89,1978). In this study, HPV-RV was further characterized in comparison with RV.

HPV-RV adsorbed more efficiently to BHK and HeLa cells and grew to high titers as compared with RV. Electron microscopic observation showed that HPV-RV resembles RV morphologically and is approximately 100 nm in diameter whereas RV and latent virus with radial nucleoid are 70 and 120 nm, respectively. In order to demonstrate the common antigens between them, indirect immunofluorescent staining was carried out. Consequently, RV- and HPV-RV-infected BHK cells reacted not only with their homologous but also with heterologous antiserum. By immunoprecipitation followed by SDS-PAGE, at least two viral polypeptides (67 K and 47 K) were recognized as cross-reacting polypeptides between them. Therefore, HPV-RV was shown to contain partly structural proteins of RV.

OP17.5

COMPARISON OF THE NUCLEOTIDE SEQUENCE OF THE STRUCTURAL GENES OF A SOUTH AMERICAN STRAIN OF YELLOW FEVER VIRUS WITH VACCINE STRAIN 17D. M. Ballinger, A. Barrett, B. Miller
Division of Vector-Borne Viral Diseases, Center for Infectious Diseases, Centers for Disease Control, PHS, HHS, Fort Collins, Colorado, USA

Geographically distinct strains of yellow fever virus have been shown to vary in biological and physical properties. Specifically, studies have shown differences between South American and African YF strains in their virulence for mice and in the physical properties of their envelope glycoprotein (E). Additionally, genetic heterogeneity has been demonstrated between S. American and African YF strains by oligonucleotide fingerprinting. Differences in virulence between YF Asibi virus and its vaccine derivative, 17D, have been suggested to result from changes in the E protein deduced from nucleotide sequence comparisons.

We have conducted sequence analysis of the genomic 5' end of a sylvan S. American YF strain in order to investigate the genetic basis for variations observed between S. American and African YF strains. A cDNA clone of the structural region (C, M, E, NS1) of YF 1899/81 was constructed and sequenced. The nucleotide sequence was compared with the corresponding region of YF vaccine strain 17D RNA. Additionally, a comparison was made of the deduced amino acid sequences of the two strains in order to elucidate changes in viral proteins which may be related to virulence.

OP17.6

PERSISTENT INFECTION OF PORCINE KIDNEY CELLS WITH JAPANESE ENCEPHALITIS VIRUS. D.A. Gadkari and P.S. Shah, National Institute of Virology, Pune 411 001, India.

A persistently infected (PI) cell line of porcine kidney (PS) cells was obtained from the cells surviving the infection with Japanese encephalitis (JE) virus. The cells have been passaged 82 times. Low level (0.03 to 0.1 pfu/cell) of infectious virus was detected in PI culture supernatants throughout the course of persistence. The PI cells were totally refractory to several strains of JE, West Nile and dengue viruses but the plaquing ability of antigenically unrelated viruses was not affected. A sudden loss of restriction and selective to Nakayama strain of JE virus was observed after 67 passages of the PI cells. PI culture supernatant contained large proportion of defective interfering particles and high frequency (12%) of temperature-sensitive mutants were isolated from PI cell released virus. The ts mutants were non-lethal to adult mice by the intracerebral (i.c.) route. The surviving mice produced anti-JE virus antibodies and also accepted the i.c. challenge with the wild type virus.

Immunoprecipitation and SDS-PAGE analysis of intracellular viral polypeptides from the PI cells at different passage levels revealed quantitative rather than qualitative differences.

OP17.7

THE NUCLEOTIDE SEQUENCE OF COXSACKIEVIRUS A18. P. Horsnell, *P.D. Minor and G. Stanway. Department of Biology, University of Essex, Colchester and *NIBSC, London, UK.

Coxsackie A viruses cause a wide variety of human disease. We are interested in defining the molecular basis of their pathogenicity and determining the molecular relationship between different members of the group. Initial experiments indicated that coxsackievirus A18 is particularly interesting since virus RNA hybridized strongly to poliovirus cDNA suggesting a close relationship, yet it shares a common cellular receptor with the main group of rhinoviruses. To gain an understanding of these properties we have cloned and partially sequenced the virus genome. The sequence shows remarkable homology to those of the polioviruses, particularly at the 3' end of the genome. The capsid region is rather more diverse. We intend to investigate the virus further by constructing a full-length infectious cDNA and modifying it by oligonucleotide-directed mutagenesis.

OP17.8

STUDIES ON THE 5' NON-CODING REGION OF RHINOVIRUSES. S. Hassard, P.J. Hughes and G. Stanway. Department of Biology, University of Essex, Colchester. UK.

Human rhinoviruses are the major causative agents of the common cold and are therefore important pathogens. We wish to improve our understanding of rhinovirus molecular biology with the overall aim of defining novel targets for antirhinoviral agents. Of particular interest is the 5' non-coding region which is well conserved between rhinoviruses but whose function is unknown. We have determined the nucleotide sequence of the 5' non-coding region of several rhinoviruses including serotypes 1B, 14, 45 and 72. In each case the region is about 600 nucleotides in length and there is a high degree of homology particularly around position 150-200 and 500-600. The effect of these areas on translation of viral RNA is being determined using oligonucleotide-directed mutagenesis of DNA inserted into a vector carrying a T7 promoter. Transcribed RNA is compared by translation in vitro.

OP17.9

STUDIES ON THE MOLECULAR INTERACTION BETWEEN HUMAN RHINOVIRUSES AND CELLS. R.J. Colonno, J. Tomassini, P. Callahan, S. Mizutani, J. Condra and D. Graham. Merck Sharp & Dohme Research Laboratories, West Point, PA 19486 U.S.A.

Human rhinoviruses (HRVs) are members of the picornaviridae family and are the major causative agent of the common cold in man. Competition binding and immunological studies have suggested that 88 HRV serotypes can be divided into only two groups based on receptor specificity. Monoclonal antibodies have been isolated which completely block the attachment of the "major group" (90%) of HRV serotypes. The anti-receptor antibodies have a strong avidity for the receptor and have the ability to displace previously-bound HRV virions. The "receptor blockade" concept has been tested in chimpanzees and human volunteers with encouraging results.

A 90 kDa cell surface protein was isolated by immunoaffinity chromatography. The receptor protein is an acidic glycoprotein containing sialic acid. Prolonged treatment of cells with anti-receptor antibodies had no observable effect on cell growth and division. Antibody treated cells remained protected for several days.

A series of mutants were generated by site-directed mutagenesis of an infectious HRV-14 cDNA clone. The resulting clones appear to have altered binding phenotypes and represent the first evidence that the virion canyon is involved in cell receptor interaction.

OP17.10

EFFECT OF TUNICAMYCIN ON THE REPRODUCTION AND MORPHOGENESIS OF TICK-BORNE ENCEPHALITIS VIRUS IN CELL CULTURE. M.B.Korolev, V.M.Lisak, T.I.Dzhivanyan, G.G.Karganova, V.I.Lashkevich. Institute of Poliomyelitis and Viral Encephalitides Academy of Medical Science, Moscow, USSR.

Pig embryo kidney cells infected with tick-borne encephalitis virus were exposed to the tunicamycin. We found that the selective blocking of V3 protein glycosylation took place and yields of extracellular infectious virus were also reduced by 0,25-1,0 µg/ml tunicamycin. The inhibition of glycosylation didn't disturb however the processes of assembly of virions in infected cells. At the same time in drug-treated cells there was the appreciable accumulation of virus particles in cisternaes and vacuoles of Golgi apparatus and reduction of extracellulary revealed virions. It is suggested that inhibition of glycosylation results in the disturbance of regulation on the terminal stages of transport of virus particles and its realisation from infected cell. Data obtained indicate also the important role of Golgi apparatus in the final stages of flavivirus morphogenesis.

OP17.11

IMMUNOGENICITY AND FIELD TRIAL OF INACTIVATED JAPANESE ENCEPHALITIS BEIJING-1 VACCINE. T.Kitano, S.Yabe, M.Kobayashi, A. Oya(NIH), K.Fukai(A.B.M.), T.Ogata, T.Nakamura(Teikyo Univ.), M.Minamidani(Komagome Hosp.), Y.Takeuchi(Kawasaki Hosp.), K. Ueda(Kyushu Univ.), I.Kitamura(Kochi Sch.Med.) and M.Kimura (Tokai Univ.), Tokyo, Japan.

A new Japanese encephalitis vaccine, Beijing-1 vaccine was developed. By NT test using mice sera vaccinated with Beijing vaccine or the current Nakayama vaccine and passive immunization test in mice, the efficacy of Beijing vaccine was suggested to be the same level or superior to Nakayama-Yoken vaccine.

In the field trial, 199 paired sera were obtained. Of 199, 121 sera were pre-negative and NT titer against Beijing strainor Nakayama strain was examined. After the primary vaccination, the seroconversion rate against Beijing strain reached 98% and the average NT titer was 2.86 and against Nakayama strain were between 83 to 90% and 2.39, respectively. By the booster vaccination after one month, the seroconversion rate was 100% and the average NT titer increased to 3.55 against Beijing strain. On the other hand, against Nakayama strain, though the seroconversion rate incresed between 89 to 97%, the average NT titer did not so increase and was 2.52. By the booster vaccination of one year later, NT titer against Nakayama strain also increased though the titer was not so high compared to that against Beijing strain.

OP17.12

MOLECULAR CLONING AND CHARACTERIZATION OF THE COXSACKIEVIRUS A9 GENOME. T.L. Jones, L.L. Marnell, Division of Biomedical Research, Lovelace Medical Foundation, Albuquerque, New Mexico, USA.

RNA, isolated from Coxsackievirus A9, was used as a template for cDNA synthesis using an oligo(dT) primer and reverse transcriptase. Poly(dC)-homopolymeric tails were added to the cDNA:RNA hybrids which were then annealed to oligo(dG)-tailed pBR322 and used to transform competent E.coli. A plasmid was isolated that contained a 1.8 kilobase insert. This insert was shown to be specific for Coxsackievirus A9 by dot blot hybridization analysis. The DNA of the insert was mapped using single and double digestions with several restriction endonucleases. This insert will be used to make primers in order to extend the cDNA copy to include the entire genome. In addition, dot blot hybridization experiments will be performed in order to examine the degree of genomic homology among the enteroviruses using fragments of Coxsackievirus A9 as probes.

OP17.13

CIS-ACTING ELEMENTS WITHIN THE 5' NONCODING REGION OF POLIOVIRUS mRNA ARE RESPONSIBLE FOR CELL-TYPE SPECIFIC AND CAP INDEPENDENT TRANSLATION. J. Pelletier, G. Kaplan, V. Racaniello and N. Sonenberg. Department of Biochemistry, McGill University, Montréal, Québec, Canada H3G 1Y6 and Department of Microbiology, Columbia University, New York, N.Y. USA 10032.

Poliovirus mRNA is naturally uncapped and must translate by a cap-independent mechanism. To elucidate the manner by which ribosomes initiate translation on poliovirus mRNA we have constructed a series of deletion mutants within the 5' noncoding region of the mRNA. In vitro synthesized transcripts from the SP6 promoter were assayed in several translation systems. We found striking differences in the translational efficiencies of the poliovirus mRNAs depending on the translation system used. Translation was efficient in HeLa and L-cell extracts, but was very inefficient in reticulocyte lysates, wheat germ extracts and Xenopus oocytes. We have identified within the poliovirus 5' noncoding region a cell-specific negative regulatory element of translation and an internal sequence responsible for conferring cap-independent translation. We have studied the role of the AUG codons located upstream from the major AUG initiator in translation by site directed mutagenesis. Changing these AUGs to UUGs (one at a time) had no effect on the in vitro translational efficiency of CAT mRNA fused to the polio 5' noncoding region.

OP17.14

LABORATORY DIAGNOSIS OF FETAL RUBELLA INFECTION.
L.Ho-Terry, G.M.Terry, P.Londesborough.
Department of Medical Microbiology and Department of Chemical Pathology, Faculty of Clinical Sciences, University College London, U.K.
Diagnosis of fetal rubella infection in the first trimester of pregnancy was made using either nucleic acid hybridization, immunoblot and virus isolation techniques on aborted and biopsied material or maternal IgM detection. In a limited number of 10 cases, hybridization with our cloned rubella cDNA probe and virus isolation in VERO cells showed total agreement whereas 2 potentially false positive results were recorded with immunoblot using a mixture of rubella E1 monoclonal antibodies. Fetal rubella infection was accurately diagnosed by the presence of maternal rubella specific IgM in only 7 out of 10 cases. There were 2 false positives and 1 false negative. Using a combination of techniques based on different principles, a more accurate diagnosis of fetal rubella infection can be obtained.

OP17.15

POLIOVIRUS PROTEIN VARIATION DEMONSTRATED BY 2D-PAGE.
P. Cash. Department of Bacteriology, University of Aberdeen, Aberdeen, Scotland.

Polioviruses, representing all three virus serotypes, were collected from patients receiving attenuated polio-vaccine. The proteins of these viruses and the vaccine virus were compared by high resolution 2D-PAGE. The proteins were prepared from virus-infected cells pulse-labelled for 90 min, and resolved by a combination of IEF and SDS-PAGE. Similar profiles were found for viruses within a serotype, although there were characteristic differences between the virus serotypes. No differences in the protein profiles were found between either serotype 2 or serotype 3 isolates and the respective vaccine viruses. However, two groups of serotype 1 viruses were observed among 13 type 1 isolates. These were differentiated on the basis of the charge of VP3; 9 type 1 viruses had the same profile as type 1 vaccine virus and 4 viruses had a more acidic VP3, similar in migration to VP3 of the Brunenders and Mahoney type 1 strains. The shift in mobility of VP3 was also demonstrated in serial isolates collected from a vaccinated child. The altered mobility of VP3 correlated with a change in the antigenicity of the neutralising epitope present on VP3; viruses with the acidic form of VP3 were antigenically distinct from the type 1 poliovaccine virus.

OP17.16

THE ORGANISATION AND EXPRESSION OF THE COAT PROTEIN GENE OF WHITE CLOVER MOSAIC VIRUS. S-A. HARRISON, R.L.S. Forster and R.C. Gardner. Department of Cell Biology, University of Auckland, Auckland, New Zealand and Plant disease Division, Department of Scientific and Industrial Research, Auckland, New Zealand.
White clover mosaic virus (WClMV) causes a large reduction in the nitrogen-fixing ability and the dry matter production of white clover, a major component of New Zealand's pastures. No naturally occurring resistance is known to the virus. The nucleotide sequence of the genomic RNA has been determined by dideoxy-sequencing of cDNA clones and by direct RNA sequencing. The coat protein gene has been located in the 3' portion of the genome and the subgenomic mRNA encoding the viral coat protein in vitro has been mapped to the viral RNA. The coat protein amino-acid sequence was found to be 40% homologous to that of potato virus X, the type member of the group to which WClMV belongs.
Expression of the coat protein gene in vitro by construction of appropriate cDNA clones incorporating a T7 RNA polymerase promotor and subsequent translation of the RNA transcripts confirmed the validity of the cDNA clones prior to in vivo expression work, in which the coat protein gene will be transferred to Nicotiana clevelandii in an attempt to produce plants resistant to further viral infection.

OP17.17

USE OF 5' SEQUENCES FROM POSITIVE STRAND RNA PLANT VIRUSES AS TRANSLATIONAL ENHANCERS IN VITRO AND IN VIVO. D.E. Sleat[1], D.R. Gallie[1], J.W.Watts[2], P.C. Turner[3], T.M.A.Wilson[1]. Dept. of Virus Research[1] and Cell Biology[2], John Innes Institute, Colney Lane, Norwich NR4 7UH, U.K.; Dept. of Biochemistry[3], University of Liverpool, P.O. Box 147, Liverpool L69 3BX, U.K.

A 67-nucleotide portion of the non-coding, 5'-leader sequence of tobacco mosaic virus RNA (Ω ') has been shown to enhance the translation of contiguous foreign gene transcripts both in vitro and in vivo. Chemically-synthesised Ω' was inserted into derivatives of an in vitro transcription plasmid (pSP64) between the SP6 promotor and sequences coding for chloramphenicol acetyltransferase (CAT) or neomycin phosphotransferase (NPTII). Run off transcripts, with or without a 5'-cap ($G^{5'}ppp^{5'}G$) and/or the Ω' sequence, were tested in mRNA-dependent cell-free translation systems derived from rabbit reticulocyte lysate, wheat germ extract or E. coli (MRE 600). In most cases, the presence of Ω' increased the translational expression of both genes, typically between 3- to 10-fold. The presence of non-coding RNA sequences 5' to Ω' reduced its stimulatory effect in vitro. Two Ω' sequences in tandem further enhanced expression of NPTII, compared to a single Ω' only in the E. coli in vitro translation system.

Electroporation of N. tabacum protoplasts or microinjection of X. laevis oocytes with transcripts containing the CAT coding region confirmed the potential of the Ω' sequence as an enhancer of gene expression in vivo.

OP17.18

GENETIC VARIABILITY IN NATURAL POPULATIONS OF AN RNA PLANT VIRUS. F.García-Arenal and E. Rodríguez-Cerezo. Dpt. of Plant Pathology, E.T.S.I.Agrónomos, 28040 Madrid, Spain.

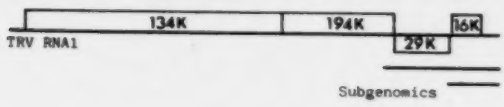
U5 strain of tobacco mosaic virus (U5-TMV) endemically infects the perennial wild plant Nicotiana glauca Grah. in dry areas of the world, providing a good system to study the genetic variability of an RNA plant virus. The genomic RNAs of U5-TMV isolates obtained from infected N.glauca plants in different parts of SE Spain were undistinguishable by molecular hybridization and S1 analysis. A more detailed comparison was done by two dimensional electrophoresis of RNase T1-generated oligonucleotides (T1 fingerprints). A difference of 2 to 20 spots (out of 60) was found between the fingerprints of any two clones representing different isolates. This means that variation occurs in 0.2-2.0 % of the positions of the RNA. These data are in the range of those found for animal RNA viruses of similar genomic size.

OP17.19

A COMMON VIRAL ANCESTOR FOR TRV AND TMV. W.D.O. Hamilton, M. Boccara* and D.C. Baulcombe. Plant Breeding Institute, Maris Lane, Cambridge CB2 2LQ, U.K. and (*) Path. Veg. I.N.R.A., 16 rue Claude Bernard, 75005 Paris, France.

The figure below summarises the genomic organisation of tobacco rattle virus (TRV) RNA1 as determined by sequence analysis of an overlapping set of cDNAs, or primer extended cDNAs for the 5' terminal nucleotides. There are extensive homologies with tobacco mosaic virus (TMV) in the general pattern of gene organisation, the use of translational readthrough (TRV has a leaky UGA stop codon between the 134k and 194k proteins) and also the derived protein sequences of the 194k and 29k proteins. Together these suggest a common viral ancestor for the two viruses.

Analysis of the 5' terminal sequence of RNA1 and RNA2 of various TRV strains suggests that viral RNAs can acquire new genes and that this even occurs quite frequently (in an evolutionary sense). This suggest a mechanism by which the tobra and tobamoviruses may have diverged from each other.



OP17.20

IN VITRO TRANSCRIPTION AND TRANSLATION OF CLONED cDNA OF CMV RNA 3. J. Owen and P. Palukaitis, Dept. Plant Pathology, Cornell University, Ithaca, NY 14853.

Cucumber mosaic virus is an isometric plant virus with a tripartite genome. The replication functions are believed to be encoded by RNAs 1 and 2, while RNA 3 contains the coat protein and may also be involved in promoting cell-to-cell movement. RNA 3 of CMV is a template for the in vitro translation of a 37K protein and also contains the sequence information for subgenomic RNA 4; the mRNA for the coat protein.

Full length cDNA to RNA 3 of the Fny strain of CMV has been cloned into pUC18 and restriction mapped. This cDNA has also been inserted into a transcription vector between bacterial promoters T_3 and T_7, and full-length RNA transcribed in vitro. RNA transcribed from the cloned DNA by T_7 polymerase annealed to the viral RNA and was resistant to digestion by mung bean nuclease, demonstrating that the RNA transcribed in vitro is colinear with the viral genome. RNA transcribed by T_3 polymerase is of the same polarity as viral RNA and in vitro translation of this RNA produced proteins of the expected size.

The ability of the in vitro RNA 3 transcripts to participate in productive infection will be discussed, as will the progress in the construction of transgenic tobacco plants with subclones of the RNA 3 clone.

OP17.21

GENOME ORGANIZATION OF MAIZE CHLOROTIC MOTTLE VIRUS. R.C. Nutter and S.A. Lommel. Stauffer Chemical Co., Richmond, CA 94804 and Dept. of Plant Pathology, Kansas State University, Manhattan KS, 66506.

Corn lethal necrosis, a severe disease of corn, is caused by the synergistic interaction of maize chlorotic mottle virus (MCMV) and either the ubiquitous maize dwarf mosaic virus or wheat streak mosaic virus. We have characterized MCMV in order to better understand the molecular basis of the disease. MCMV is a 30nm icosahedral plant virus composed of 180 identical capsid protein subunits of 24.6kd and a single genomic (+) sense ssRNA of ~4kb. In vitro translation analysis of viral RNA yielded discrete polypeptides of 50, 44, 40, 32, and 24.6kd. Immunoabsorption of the translation products with capsid protein antiserum identified the 24.6kd product as the capsid protein and indicated that it is synthesized from a subgenomic RNA in vivo. cDNA clones have been constructed representing the MCMV genome. Several large open reading frames have been identified by sequence analysis. The capsid protein ORF has been mapped to the 3' end of the genomic RNA. Further sequencing as well as translation of cloned transcripts are being employed to further determine the genome organization.

OP17.22

NUCLEOTIDE SEQUENCE ANALYSIS OF WHITE CLOVER MOSAIC VIRUS. R.L.S. Forster[1], M. Bevan[1,2], S-A. Harbison[3] and R.C. Gardner[3]. [1]Plant Diseases Division, DSIR, Private Bag, Auckland, New Zealand, [2]Plant Breeding Institute, Cambridge CB2 2LQ, UK, [3]Cell Biology Dept, University of Auckland, Auckland, New Zealand.

The genome of the potexvirus white clover mosaic virus is a positive-sense single-stranded RNA with a 3'-terminal poly(A) tract. The complete sequence of 5844 nucleotides has been determined from cDNA clones. Three open reading frames (ORF) have been found. The largest ORF begins 108 nucleotides from the 5' terminus and correlates with an *in vitro* translation product of mol. wt. 160,000 (160 K). An internal ORF correlates with a translation product of 37 K. A third ORF, near the 3' terminus, is the likely virus coat protein.

OP17.23

BEAN MILD MOSAIC VIRUS. V.Hari, I.P.S. Gadh, D. Baunoch. Dept. of Biological Sciences, Wayne State University, Detroit, USA.

Bean Mild Mosaic Virus is an isometric, unclassified, beetle-transmitted RNA plant virus. Virus particles are 28nm in diameter, sediment at 127S, and contain a single RNA of M.W. 1.27×10^6. Experiments carried out in my laboratory show that virus extracts contain two RNAs of M.W. 1.5×10^6 and another of M.W. 0.76×10^6. Of these, RNA I alone is infectious. c-DNA probes prepared using RNA I or II (0.76×10^6) cross hybridized to both RNA I or II, and it appears that RNA II is an encapsidated subgenomic RNA.

In vitro translation of BMMV leads to synthesis of proteins of M.W. 89K, 75K, 57K, 43K, 40.5K, 37K, 25K, 23K, and 20.5K. Of these, the 40.5 and 25K proteins are the major translation products. Pulse chase experiments do not indicate polyprotein processing. The two major proteins are early translation products, but we have no information as yet on whether both or one of these is coded by the subgenomic RNA. Peptide mapping of the translation products, c-DNA cloning, and sequence data will be presented.

OP17.24

GENE FUNCTION IN COWPEA MOSAIC VIRUS. C. Holness, G.P. Lomonossoff and D. Evans. Department of Biological Sciences, University of Warwick, Coventry, Great Britain.

Cowpea mosaic virus (CPMV) is one of the best characterized plant viruses. The nucleotide sequence of its bipartite, positive sense single-stranded RNA genome has been determined. The CPMV replication strategy involves the synthesis of a polyprotein precursor from each of the RNAs which are cleaved into the functional proteins by virus encoded proteins.

The location on the genome of the nucleotide sequences encoding the coat proteins, the RNA polymerase, the proteases and the VPg have been identified. The functions encoded by approximately a third of the genome remains to be elucidated. The approach we have taken to determine these functions is the isolation and characterization of temperature-sensitive mutants. A number of mutants has been isolated by nitrous acid mutagenesis and their phenotype, in both infected plants and protoplasts, will be discussed. The site of mutagenesis has been identified by 2D-RNA fingerprinting and primer extension sequencing.

OP17.25

BIOSYNTHESIS OF CUCUMBER MOSAIC VIRUS-ENCODED POLYPEPTIDES IN VIVO. A Kaminski, K.R.Wood, Department of Microbiology, University of Birmingham, Birmingham B15 2TT, U.K.

The genome of CMV consists of 3 ss (+) RNA strands (1-3), which produce proteins of approximate molecular weights (M_r) of 110, 95 and 30,000 (kD) respectively when translated in vitro in reticulocyte lysates. The capsid protein M_r ca 26 kD, is translated from a fourth RNA species, the sequence of which represents the 3' terminus of RNA 3.

Actinomycin D and chloramphenicol inhibited cucumber mesophyll protoplasts and tobacco leaf strips were infected with CMV RNA or virus particles. Proteins synthesised after infection were pulse-labelled with ^{35}S-methionine for various times, electrophoretically separated and examined by fluorography and also serologically. Infection-specific proteins found in protoplasts had M_r 102, 26, 20 and 18 kD, while those found in leaf strips had M_r 37, 30, 26, 20, 18 and 16 kD. So far, two of these proteins, 102 kD and 26 kD, have been found to be serologically related to the translation products of CMV (W) RNA 2 and RNA 4 respectively. The 30 kD protein is presumed to be the RNA 3 translation product. The others may result from post-translational modifications of CMV-encoded proteins.

OP17.26

MAPPING EPITOPES IN THE E PROTEIN OF JAPANESE ENCEPHALITIS VIRUS. P. W. Mason*, M. J. Fournier and T. L. Mason. Dept of Biochemistry, Univ. of Massachusetts, Amherst, MA 01003, USA, Present address, YARU, Yale Univ. School of Medicine, New Haven, CT 06510, USA.

We have expressed segments of the JE (Nakayama) E protein in *E.coli* and have shown that the recombinant proteins react with anti-JE hyperimmune ascitic fluid (HMAF) and with 10 anti-E MAbs. Initially, two antigenic regions in E were identified by screening λgt11 expression libraries. One region lies near the N-terminus and is reactive with the antisera but not with any of the MAbs; the second is in the C-terminal half of the protein and reacts with both the polyclonal sera and each of the 10 MAbs. These MAbs bind to topographically related but distinct epitopes on the surface of the virus; four of them efficiently neutralize the virus and two others are flavivirus cross-reactive. All 10 epitopes are localized between amino acid residues 280 and 400 of the mature protein. Preliminary efforts to dissect this region by progressive exonuclease digestion of cDNA fragments have failed to yield a significantly shorter protein segment that will still bind any of the MAbs. *In vitro* mutagenesis is now being used to generate variants capable of discriminating among the MAbs.

OP17.27

INFECTIOUS Q8 BACTERIOPHAGE ELICITED BY NEGATIVE-STRAND TRANSCRIPT RNAs. P. Shaklee, J. Miglietta, A. Palmenberg, and P. Kaesberg, Biophysics Laboratory, University of Wisconsin, Madison, Wisconsin, U.S.A.

A plasmid, pQ8, containing a full-length cDNA copy of the Q8 RNA phage genome with flanking T3 and T7 promoters was constructed. Positive-strand Q8 RNA, generated by in vitro transcription of the cloned DNA with T3 RNA polymerase, was infectious to E. coli spheroplasts. The Q8 replicase gene from pQ8 was subcloned into a thermo-inducible vector and expressed in E. coli cells. Full-length negative-strand Q8 transcripts, made with T7 RNA polymerase, were infectious only when transfected into spheroplasts containing the induced viral replicase. Recent experiments have shown that the plasmid encoded replicase can fully complement replicase function in transcript RNAs altered in the replicase gene. Transfection of these altered RNAs into spheroplasts containing the induced replicase yields virus which form plaques only on lawns of E. coli cells also expressing the replicase plasmid. Small insertions and deletions within the replicase gene were found to be allowable mutations and current work is designed to assess the viability of larger alterations within this cistron.

OP17.28

GENOMIC RNAs OF BROME MOSAIC VIRUS AND COWPEA CHLOROTIC MOTTLE VIRUS: MOLECULAR CLONING, SEQUENCE HOMOLOGIES AND ACTIVITY OF CHIMERIC RNAs. Jozef J. Bujarski, Aleksandra Dzianott and Paul Kaesberg, Biophysics Laboratory and Dept. of Biochemistry, University of Wisconsin, Madison, WI 53706, USA.

Three closely related bromoviruses, brome mosaic virus (BMV), cowpea chlorotic mottle virus (CCMV) and broad bean mottle virus (BBMV) differ dramatically in their host range. To analyze how virus-encoded genetic information affects biological properties of these viruses, we now analyze the genomic RNAs of CCMV and BBMV and compare with previously analyzed BMV RNAs.

We have completed the sequence of cDNA clones of CCMV RNA1 which shows about 70% homology on the nucleotide level with BMV RNA1. Like BMV, CCMV RNA1 is monocistronic and encodes for 958-amino acid long polypeptide homologous by 75% to BMV RNA1 polypeptide suggesting similar function for both proteins. Currently we are sequencing cDNA clones of CCMV RNA2.

In order to analyze the role of individual domains of CCMV and BMV RNA1 for virus infectivity, we are constructing chimeric cDNA1 molecules where individual regions in BMV cDNA1 infectious clone are substituted by homologous cDNA fragments from CCMV cDNA1 (and vice versa) without changing the open reading frame. The effect of these substitutions on biological activity of transcribed chimeric RNA1 molecules in protoplasts and whole plants will be discussed.

OP17.29

A NEW MOSQUITO CELL-LINE FOR USE IN ARBOVIROLOGY M.J. Oelofsen[1], M.S. Smith[1] and T.C. de K. van der Linde[2]. Departments of Medical Microbiology (Virology)[1] and Entomology[2], University of the Orange Free State, Bloemfontein, South Africa.

As the mosquito Culex theileri is probably the major vector of Rift Valley fever (RVF) virus during epizootics on the inland plateau of Southern Africa, a cell line has been developed from the tissues of this arthropod to facilitate laboratory studies on RVF virus and other arboviruses.

Temperature pretreatment of egg rafts before initiation of cultures markedly affected both the cell type and growth of cells, while the pH of the growth medium was equally important. It was also easier to establish cultures using preconditioned culture media.

Parameters of optimal growth of the cell line, such as fetal calf serum concentration and growth tempo, have been established. To characterise the cell line and distinguish it from other established arthropod cell lines used in this laboratory, namely, Igarashi's C6/36 clone of Aedes albopictus cells and the C17 (A. aegypti) cell line, several studies have been performed. These include free fatty acid analyses, cytogenetic studies, isoenzyme analyses and the ability or not of arboviruses (RVF, West Nile and Sindbis viruses) to replicate or to cause a visible cytopathogenic effect. These studies reveal beyond doubt that this is a new cell line and not a contaminant from the other lines.

OP17.30

AGE DEPENDENT DIFFERENTIAL INFECTION OF PRIMARY BRAIN CELL CULTURES BY THE AVIRULENT STRAIN OF SEMLIKI FOREST VIRUS A7. C.B. BRUCE*, J.A. Chapman*, M.G. Rumsby*. *MRC Common Cold Unit, Harvard Hospital, Salisbury SP2 8BW U.K. *Department of Biology, University of York, York YO4 5DD U.K.

Semliki Forest Virus (SFV) is a neurovirulent virus known to cause demyelination in rodents. Using primary cell cultures made from cerebral hemispheres of neonatal (1-2 day old) rat pups we have investigated the effect of A7 SFV in vitro. It has been shown using immunocytochemical techniques that, at 10 days in vitro, cells from the A2B5 cell lineage (ie progenitor glial cells, type II astrocytes and oligoentdrocytes) are rapidly infected and killed 8-10h pi. The predominant cell type present in the cultures, type I astrocytes, do become infected but a c.p.e. is not observed until 24-30h pi if the primary cultures are allowed to mature further and infected at 3 weeks in vitro the oligodendrocytes are again infected first and have been removed from the cultures by 24h pi. The type I astrocytes however remain, showing no signs of c.p.e. upto 80h pi. The virus titre obtain from the 3 week old cultures increases by 1-2 log compared with a 4 log increase in the 10 day old cultures. Although these 3 week old cultures are metabolically less active than the 10 day old cultures this does not appear to be the reason for their lower infectivity.

OP17.31

ISOLATION OF A NOVEL RETROVIRUS FROM BOVINE MATERIAL.
A-L Williamson, D.F. York, B.J.H. Barnard and D.W. Verwoerd, Veterinary Research Institute, Onderstepoort, 0110 South Africa.

A novel retrovirus was isolated from lymphocytes obtained from a cow with malignant catarrhal fever. This "bovine" retrovirus (BRV) replicates in calf thymus (CFTH) cells with the formation of syncytia. When infected cells were injected subcutaneously into nude mice, tumours were visible within 10 days. No tumour formation was observed with uninfected CFTH cells indicating that BRV is a transforming retrovirus.

The virus has a density of 1,16 in isopycnic sucrose gradients. Its morphogenesis is similar to that of type D oncoviruses i.e. there are intra-cytoplasmic particles which usually bud with a complete core to form immature particles. The mature particles have a centrally located nucleoid. Negatively stained particles have no surface spikes.

Using an immunoblot assay, BRV crossreacts serologically with Mason Pfizer monkey virus but not with Maedi-Visna virus or bovine leukemia virus.

OP18.1

APPEARANCE OF FC (IGG) RECEPTOR(S) IN HUMAN EMBRYONIC LUNG FIBROBLASTS INFECTED WITH SIMIAN CYTOMEGALOVIRUS.

Y. EIZURU, Y. MINAMISHIMA. Department of Microbiology, Miyazaki Medical College, Kiyotake, Miyazaki, Japan.

Human embryonic lung fibroblasts (HEL) infected with simian cytomegalovirus (CMV) were found to bind nonspecifically Fc portion of human IgG. Binding of IgG to simian CMV-infected HEL, but not to uninfected HEL, was visualized as the cytoplasmic fluorescence by indirect immunofluorescence test, but not related to the presence of antibodies against human CMV. The reaction was positive in the combination of purified IgG and Fc, but not Fab, fragments with anti-IgG, but not anti-IgM or IgA, fluoresent conjugates. IgG bound to the receptor did not fix complement (C), when tested by anti-C3 immunofluorescence test. The Fc receptors were induced by 4 simian CMV strains so far tested: a laboratory strain originally isolated from an African green monkey (GR2757), an isolate from primary culture of African green monkey kidney cells (QV3), and two fresh isolates from wild Macaca monkeys in Japan (MV1 and MV2). Sera of monkey, goat, rabbit and mouse were also reacted with the Fc receptor.

Thus, simian CMV as well as human CMV induces in HEL receptor(s) for Fc (IgG) of different species.

OP18.2

SYNERGISTIC INHIBITION OF ANATID HERPESVIRUS REPLICATION BY ACYCLOVIR AND PHOSPHONO-COMPOUNDS J. Johnson, N. Attanasio, Discipline of Microbiology, University of Osteopathic Medicine and Health Sciences, Des Moines, Iowa, U.S.A.

Anatid herpesvirus, the causitive agent of duck plaque, a necrotic enteritis of anseriformes, has been responsible for two major epizootics and several minor outbreaks in the U.S.A. since its identification in captive waterfowl on Long Island, N.Y. during the winter of 1967. Because of the continuing sporadic occurrence of duck plaque and the possibility of Anatid herpesvirus latency, we have explored the replication of the virus in the presence of several inhibitors. Plaque reduction assays were used to evaluate the inhibitory effects of acyclovir (ACV), phosphonoacetate (PAA), and phosphonoformate (PFA) with a plaque purified isolate of Anatid herpesvirus (AHV-ppc3). A plaque assay employing a liquid overlay medium was developed to facilitate the drug inhibition studies. From dose-response curves, the ID_{50} for PAA, PFA and ACV were 20 µg/ml, 12 µg/ml and 0.14 µg/ml, respectively. From data obtained from combination dose-response curves, dose isobolograms were prepared and used to determine the types of interaction between drug pairs. Whereas the interaction between PFA and PAA was additive, synergism occurred with ACV and either PAA or PFA. Drug resistant mutants of AHV-ppc3 resistant to 8.0 µg/ml ACV, 250 µg/ml PAA, 180 µg/ml PFA or 6.0 µg/ml AHV and 220 µg/ml PAA were isolated.

OP18.3

COMPARISON OF CYTOMEGALOVIRUS ISOLATES FROM WILD CAUGHT MICE AND RATS WITH MONOCLONAL ANTIBODIES AND RESTRICTION ENDONUCLEASE MAPPING. A.Osterhaus, J.Groen, I.Spijkers, C.Bruggeman, I.Claassen. National Institute of Public Health and Environmental Hygiene, Bilthoven, The Netherlands.

Ten virus isolates from apparently healthy mice (M.musculus; n=5) and rats (R.norvegicus; n=5) caught at different locations in The Netherlands, were characterized as mouse cytomegaloviruses (MCMV) and rat cytomegaloviruses (RCMV) respectively, on the basis of cytopathic changes in vitro, morphology in negative contrast electron microscopy and serological cross-reactivities. The reactivities of the viruses with a panel of anti-MCMV and anti-RCMV monoclonal antibodies were tested in virus neutralization and immunofluorescence tests. Their genomes were compared by restriction endonuclease mapping. The isolates exhibited different specificities with the panel of monoclonal antibodies and showed different restriction patterns

OP18.4

PRODUCTION AND CHARACTERISATION OF MONOCLONAL ANTIBODIES TO SUBTYPE 1 AND SUBTYPE 2 VIRUSES OF EQUID HERPES VIRUS TYPE 1. R.F. Cook, R. Sinclair, J.A. Mumford. Equine Virology Unit, Animal Health Trust, Nr. Newmarket, England CB8 7PN.

Monoclonal antibodies have been prepared to subtype 1 (abortion) and subtype 2 (respiratory) viruses of Equid Herpes Virus Type 1 (EHV-1) by fusing spleen cells from immunized mice with the myeloma cell line NS0. Twenty-eight stable hybridoma lines secreting antibody to EHV-1 have been generated of which sixteen were produced against the subtype 1 strain A183 and twelve against the subtype 2 strain MD. Analysis by ELISA and radioimmunoassay has shown that four of the subtype 1 and six of the subtype 2 hybridomas are cross-reactive between MD and A183.

Studies are in progress using immunoblotting to determine the target antigens of each monoclonal antibody and so identify subtype specific and subtype cross-reactive epitopes. To date, a subtype 2 specific epitope has been identified on a 96kDa glycoprotein and subtype cross-reactive epitopes on 70kDa and 10kDa polypeptides. One monoclonal antibody has been identified which reacts with two polypeptide bands which have approx. mw of 200kDa and 70kDa.

OP18.5

THE SPREAD OF EQUINE HERPESVIRUS ABORTION.

M. Sabine, L. Herbert. Department of Veterinary Pathology, Faculty of Veterinary Science, University of Sydney, N.S.W., Australia.

Equine herpesvirus 1 (EHV 1) causes respiratory infection, abortion and occasionally neurological signs. Epidemic abortion caused by a herpesvirus was first recognized in Australia in N.S.W. in 1977. Minor outbreaks have occurred in the intervening years. In 1986 there were severe "abortion storms" on two studs in N.S.W. resulting in the loss of over 50 foals. The conventional explanation of respiratory spread of virus from weanling foals to brood mares did not fit the facts. The possibility of persistent or intermittent shedding of virus from carrier animals is being investigated.

Restriction enzyme analysis of DNA revealed no change in pattern between 1977 viruses and those isolated in subsequent years.

OP18.6

ANALYSIS OF THE INTERACTIONS BETWEEN BOVINE HERPESVIRUS 1 AND CATTLE BY KINETIC LOGIC.

E. Thiry, P.-P. Pastoret, R. Thomas.
Service de Virologie - Immunologie et Pathologie des maladies virales, Faculté de Médecine vétérinaire, Université de Liège, B-1070 Bruxelles et Département de Biologie moléculaire, Université Libre de Bruxelles, B-1640 Rhode St Genèse, Belgique.

Description of the interactions between bovine herpesvirus 1 (BHV-1) and its host was performed by kinetic logic, a method using boolean algebra. Each variable can take two possible values, 1 and 0, which tell whether an element is present or not at a significant level. A logical variable and a logical function are associated to each element of the system. Specific delays of appearance and disappearance are given for each logical variable.
The consequences of the persistence of latent BHV-1 in bovine were detailed in a dynamic analysis.
This logical formalization, even though schematic, allows us to represent the facts experimentally observed. It suggests answers for results difficult to interprete and gives predictions which can be experimentally tested. Logical analysis can be considered as a tool in describing the essential aspects of a complex system such as latent infection by BHV-1.

OP18.7

BIOLOGICAL AND BIOCHEMICAL COMPARISON OF BOVID HERPESVIRUS-4 STRAINS. J. Dubuisson, E. Thiry, F. Thalasso, M. Bublot, P.-P. Pastoret.
Department of Virology, Immunology and viral diseases, Faculty of Veterinary Medicine, University of Liège, Brussels, Belgium.

Bovid herpesvirus-4 (BHV-4) is a herpesvirus isolated from various clinical syndromes in cattle. The strains are usually serologically identical and other markers must be used in order to differentiate between them. The differentiation of 4 BHV-4 strains, Movar 33/63, DN599, LVR140 and V.Test was therefore attempted by the analysis of their biological and biochemical properties. BHV-4 strains were compared by several methods: cross serologic relationship studied by indirect immunofluorescence; kinetic of intracellular and extracellular viral production; comparison of the mean plaque size; restriction analysis of the viral DNA performed by the endonucleases Eco RI, Bam HI and Hind III.

As expected the 4 BHV-4 strains were serologically identical and the kinetic of intracellular and extracellular viral production was very similar. The comparison of the mean plaque size allowed to classify BHV-4 into 3 classes (class I: Movar 33/63; class II: LVR140; class III: V. Test and DN599). Restriction analysis of viral DNA revealed clear differences between the electrophoretic patterns of the 4 strains. The differentiation between BHV-4 strains can therefore be achieved by a biological method (mean plaque size) and by restriction analysis.

OP18.8

USE OF COUNTER IMMUNOELECTROPHORESIS TEST IN THE DETECTION OF EQUINE HERPES VIRUS TYPE-I ANTIBODIES AND ITS COMPARISON WITH COMPLEMENT FIXATION TEST. C. Kapoor and S.C. Tewari,
Department of Veterinary Microbiology, Haryana Agricultural University, Hisar-I25004, India

The counter immunoelectrophoresis (CIE) test was standardized for the first time for detecting equine herpes virus type-I (EHV_I) antibodies in the sera samples of aborting mares and equines showing neurological disorder. Using polyethylene glycol concentrated cell culture adapted EHV_I antigen, four lines were obtained with reference serum and positive field serum samples. Out of a total of 302 equine sera samples I50 and I43 sera samples were found positive through CIE and CF tests respectively. The CIE test showed advantage over the CF test as the seven donkey sera which were anticomplementary could be tested through this test. The test appeared to be simple, quick and less cumbersome than CF test.

OP18.9

CHARACTERIZATION OF A NEW WOODCHUCK HERPESVIRUS. E.M. Schechter[1], J. Summers[2], C.W. Ogston[1]. [1]Department of Immunology/Microbiology, Rush University, Chicago, IL, USA, [2]Fox Chase Cancer Center, Philadelphia, PA, USA.

A DNA virus with the morphological appearance of a herpesvirus has been isolated from woodchuck hepatocytes cultured in vitro. The presence of a virus was first suspected when spontaneous lysis of the cultured cells was observed. Electron microscopy of the cell supernatants showed enveloped particles with the characteristic appearance of herpesviruses. The nucleic acid isolated from purified virions is double-stranded DNA of $80\text{-}82 \times 10^6$ daltons molecular weight. The virus has been found to replicate with a lytic effect in Vero, CV-1, Owl Monkey Kidney, and Baby Hamster Kidney cells, as well as in WCH-17 cells, a line established from a woodchuck hepatoma (B. Fernie, pers. comm.). No lysis has been seen in RSC-1 cells nor in the human liver cell lines Hep G2, PLC/PRF/5 or HEL. An indirect immunofluorescence assay developed to detect antibodies to this virus in woodchuck serum has shown the presence of antibody in 4 of 18 animals in our colony. We are currently evaluating sera from woodchucks in other colonies to determine the seroprevalence of this virus in laboratory woodchucks.

OP18.10

LACK OF IgG-Fc BINDING ACTIVITY OF BOVINE HERPESVIRUSES 1 AND 2 (BHV-1 AND BHV-2). L. Rodríguez, J. Zmudzinski, and G.J. Letchworth. School of Veterinary Medicine, Heredia, Costa Rica (LR). Veterinary Institute, Pulawy, Poland (JZ) and Department of Veterinary Science, University of Wisconsin-Madison WI. USA (GJL).

The ability of BHV-1 and BHV-2 infected cells to bind radiolabelled protein A-purified rabbit and bovine IgG by the Fc portion was investigated and compared with that of HSV-1, which is known to have an Fc receptor. No IgG binding activity could be demostrated on either BHV-1 or BHV-2 infected MDBK cells. MDBK cells infected with HSV-1 bound rabbit IgG but not $F(ab^-)$ 2 fragments, demonstrating that the binding was by the Fc portion. The lack of binding of rabbit and bovine IgG by BHV-1 and BHV-2 was not affected by using primary bovine kidney or testicle cells. The binding of bovine IgG-Fc fragments was also tested. Only HSV-1 showed an increase in binding of bovine Fc in a time course experiment, whereas, BHV-1 and BHV-2 did not, despite the increase in expression of viral proteins demonstrated by the increased binding of specific antibodies. The ability of BHV-1 envelope proteins to bind rabbit and bovine IgG was tested. None of the three major envelope proteins bound to rabbit or bovine IgG.

OP18.11

ANTIGENIC CONSERVATION OF GLYCOPROTEINS AMONG PRIMATE ALPHAHERPESVIRUSES. R. Eberle and J. Hilliard. Department of Veterinary Parasitology and Microbiology, Oklahoma State University, Stillwater, OK; and Department of Virology and Immunology, Southwest Foundation for Biomedical Research, San Antonio, TX, USA.

The extent of molecular and antigenic conservation among primate alphaherpesvirus glycoproteins (gps) was evaluated. Infected cell surface gps (110-125 kd and 60-79 kd) of herpes simplex viruses, simian agent 8, and Herpesvirus simiae cross-reacted extensively, indicating conservation of structural and/or functional properties. The 110-125 kd gps of two South American primate viruses, Herpesvirus ateles type 1 and Herpesvirus saimiri type 1, also cross-reacted with the 110-125 kd gps of the other four viruses. Significantly less cross-reactivity was noted for the 75-80 kd gps of H. ateles and H. saimiri. Virion surface antigens displayed a similar pattern of reactivity. Neutralization assays and determination of neutralization rate constants confirmed these results. Immunoprecipitation was performed to identify antigenically common and unique gps. The extent of molecular conservation and antigenic structure inherent in each of these viruses reinforces previous data demonstrating close parallels between the evolutionary relationships of the natural host species and their indigenous herpesviruses.

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OP18.12

VIROLOGICAL STUDY OF VESICULAR AND ULCERATIVE LESIONS OF MOUTH AND LIPS.

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Since viruses have an important role in the etiology of ulcerative and vesicular lesions of mouth, we carried out a research concerning the eventual viral origin of those types of lesions in patients who consulted with oral medicine department of the Tehran Univeraity dentistry school from April 1985 through the end of August 1986. The sampling procedure being important for virus isolation, according to the nature and location of lesions, three different ways of sampling have been used. The specimens collected from 68 patients were inoculated simultaneously to the three different kinds of cell systems which are Hela and Vero continuous cell lines and primary cell culture of human embryo kidney. This investigation ended in isolation of 29 strains of viral agent which are divided as following: 23 strains of herpes virus type I, two strains of coxakie virus type B_3, one strain of adenovirus type 4 and three strains of non identified cytopathic agents.

OP18.13

ANTIGENIC SITES ON HUMAN CYTOMEGALOVIRUS GLYCOPROTEIN A DEFINED BY MONOCLONAL ANTIBODIES. N.Lussenhop, R.Goertz, M. Wabuke and R. Gehrz. Biomedical Research Center, Children's Hospital, St. Paul, MN 55102. A panel of thirteen monoclonal antibodies to human cytomegalovirus (HCMV) glycoprotein A was generated. These antibodies immunoprecipitated three forms of glycoprotein A under reducing conditions which had molecular weights of 130,000, 93,000, 52,000-50,000. (B. Kari,et.al., 1986 J.Virol. 60:345-352). Competitive enzyme linked immunosorbent (ELISA) assays were performed in order to study the relationship of the antigenic sites recognized by the various antibodies. Either inhibition or augmentation in the binding of the antibodies to the virus was observed when a second antibody was present simultaneously. We were able to assign the antibodies to at least five groups recognizing distinct antigenic sites. The ability of these monoclonal antibodies to neutralize HCMV Towne strain in the presence of complement was tested in a plaque reduction assay. One group, comprised of monoclonal antibodies 9B7 and 18F9, was highly neutralizing (2ug/ml for 50% plaque reduction). The binding of this group of antibodies to the virus in the ELISA assay was increased two-to four-fold in the presence of a second group of antibodies which contain antibodies that do not neutralize the virus. The neutralizing activity of 9B7 was increased by twenty-fold when a non-neutralizing antibody 41C2 from this second group was added in the plaque reduction assay.

OP18.14

CHARACTERIZATION OF HUMAN CYTOMEGALOVIRUS (HCMV) GLYCOPROTEINS.
B. Kari and R. Gehrz, BRC Children's Hosp. St. Paul, MN, USA.

We have characterized 3 immunologically distinct HCMV glycoproteins. All 3 were obtained from non-ionic detergent extracts of purified virus, suggesting an association with the viral envelope. Glycoproteins were purified by a combination of HPLC and immunoaffinity methods. Two glycoproteins, classified as gA and gB, were detected in disulfide linked complexes. The third glycoprotein, which was classified as gC, was not associated with other glycoproteins by disulfide bonds. Non-ionic detergent extracts contained multiple forms of gA and gB differing in molecular weight. However, only one form of gC was found in the same preparations. Analysis of gC and the most abundant form of gA showed that their molecular weights in SDS-PAGE and carbohydrate composition were very similar. The majority of the oligosaccharides in gA and gC were N-linked. These glycoproteins also contained similar amounts of sialic acid. Thus, gA and gC appear to follow similar routes of post translational processing. With gB there were at least 3 major forms present containing significant amounts of both N and O-linked oligosaccharides. Glycoprotein B also contained greater amounts of sialic acid as compared to gA and gC. However, the lower molecular weight forms of gB contained more carbohydrate relative to protein. Furthermore, as the molecular weight of the different forms of gB decreased the relative abundance of O-linked oligosaccharides increased. These results suggested that gB follows a considerably different route of post translational processing and that the higher molecular weight forms of gB may give rise to the lower molecular weight forms by proteolysis during or after O-glycosylation.

OP18.15

APPEARANCE OF THE SPECIFIC IgA CMV ANTIBODY AND CMV EXCRETION IN SALIVA OF INFANTS A.Tananka and Y.Numazaki, Clinical Research Division, Sendai National Hospital, Sendai, Japan.

To clarify the relationship between the cessation of the CMV excretion and the local immune response to the virus in CMV infections of infants, we studied longitudinally the appearance of the CMV specific IgA neutralizing (NT) antibody and the virus excretion in saliva with 30 healthy infants. Results obtained were as follows;
1) CMV NT antibody was measured by plaque assay after adjusting the IgA level to 20 mg/dl by freeze dry technique. CMV-NT antibody demonstrated in saliva was removed by the absorption with anti-human IgA serum but not by that with anti-IgM and IgG sera, suggesting the NT antibody in saliva belongs to the IgA class. 2) CMV-NT antibody in saliva was demonstrated in early infancy (4 month of age). However, infants having NT antibody in saliva continuously shed the virus into saliva for long time.

There was no correlation between the appearance of CMV specific IgA NT antibody in saliva and the cessation of the virus excretion to saliva in CMV primary infections of infants.

OP18.16

PURIFICATION AND CHARACTERIZATION OF A 30,000-DALTON GLYCOPROTEIN OF HSV-2. Qin Ke-feng,Wang Mei-xian,Jiang Shao-zun,Ma Wen-yu,Dept.Microbiology, 4th Military Medical College,Xian,Shaanxi,China.

A 30,000-dalton glycoprotein(g3OK) Of HSV-2 was purified by affinity chromatography.The McAb CH-A9 was used in these studies;CH-A9 has been shown by indirect ELISA and IF on fixed and unfixed HSV-infected BHK cells to be specific for a HSV-2 glycoprotein.A cell membranous extract prepared from either HSV-1 or HSV-2 infected or normal BHK cells was loaded onto an immunoadsorbent column consisting of the McAb CH-A9 linked with 3M KSCN.Only on the elution curve of the cell membranous extract prepared from HSV-2 infected BHK cells a peak could be observed.

Evidence is presented which shows that the g3OK has properties distinct from those assigned to any other HSV glycoprotein.Firstly,its molecular weight (30,000 dalton) determined by SDS-PAGE is lower than other HSV glycoproteins.Secondly,the glycoprotein is HSV-2 type specific shown in ELISA tests.Finally,this glycoprotein can induce HSV-2 type specific neutralizing antibody in BALB/c mice.

OP18.17

P3HR-1-LIKE NONTRANSFORMING VIRUS IN SALIVA AND PERIPHERAL LYMPHOCYTES FROM 2 PATIENTS WITH CHRONIC ACTIVE EPSTEIN-BARR VIRUS (EBV) INFECTION. J.M. Joncas[1], C. Alfieri[1], N. Raab-Traub[2] and J. Pagano[2]. Department of Microbiology and Immunology, Ste-Justine Hospital, Montreal, Quebec, Canada[1] and Cancer Research Center, School of Medicine, University of North Carolina at Chapel Hill, N.C., USA[2].

The saliva and oropharyngeal cell extracts of a father and his daughter, both suffering from chronic active EBV infection, contained a P3HR-1-like virus inducing EA in Raji cells and expressing EA in fresh EBV negative lymphocytes but unable to transform cord blood lymphocytes. This virus, like the P3HR-1 virus could not lead to EBV-DNA replication in contrast to the B95-8 virus. Smears of the daughter's recurrent mouth ulcers were positive for EBV-EA and VCA by IF using specific polyclonal and monoclonal antibodies. DNA extracted from these cells and digested with Bam HI showed in Southern blots a 6 kb fragment strongly homologous to the Bam HI-V (3 kbs) and B1 (9.6 kb) labelled probes. Short lived spontaneous cultures (< 1 month), two EBV B95-8 transformed lymphoblastoid cell lines from the patients and a spontaneously established cell line from the mother were overly permissive, expressing EBNA, EA and VCA. Southern blots of Bam HI and EcoRI digests of the DNA extracted from these cell lines, probed with P32 labelled Bam HI-G, H and B1 revealed, in addition to the expected B95-8 fragments, other shorter fragments of a presumably endogenous defective EB virus.

OP18.18

VARIATION IN THE NUMBER OF SITES OF LATENCY OF HERPES SIMPLEX VIRUS IN TRIGEMINAL GANGLIA OF INBRED MICE. S.Z. Abghari, J.C. Kindle, R.D. Stulting, Department of Ophthalmology, Emory University School of Medicine, Atlanta, Georgia 30322 USA.

Following topical ocular inoculation of HSV, more virus was recovered from latently infected trigeminal ganglia (TG) of BALB/c mice than C57BL/6 mice. Since these quantitative differences may reflect a difference in the number of latently infected cells or a difference in the ability of virus to replicate in secondarily infected cells, the number of sites of latency in TG of inbred mice was measured directly. At 6-8 weeks after topical corneal inoculation when no active ocular disease was present, minced TG was digested in 2% collagenase. The dissociated cells (in growth media containing 0.1% human IgG) were placed on monolayers of vero cells, incubated at 31°C, and observed for CPE for 14 days. Positive plates were stained and plaques counted. TG from BALB/c mice produced more foci of infection than TG from C57BL/6 mice. The number of foci was also dependent upon dose of virus used to infect the eye. Following infection with high doses of HSV, virus was also reactivated from contralateral ganglia, but at much lower efficiency. These data support the hypothesis that host genetic factors play a role in controlling the establishment of and/or reactivation from latency in ganglion cells. Supported by NIH EYO 5097, EYO 7092 and Heed Ophthalmic Foundation.

OP18.19

VARICELLA-ZOSTER VIRUS INFECTION OF NERVE CELLS : IN SITU DETECTION BY GENOMIC PROBES.

M.P. Herville-Louis[1], C. Sadzot-Delvaux[1], P. Delrée[2], G. Moonen[3], J. Piette[1] and B. Rentier[1] Laboratories of Microbiology[1], Histology[2] and Neurology[3], University of Liège, Belgium.

Primary infection of humans with Varicella-zoster virus (VZV) may result in a latent infection of sensory neurons.

In order to study the interactions of VZV with nerve cells, dissociated cultures of adult rat dorsal root ganglia were infected with cell-associated VZV by cocultivation and fixed at different times post infection. The presence of VZV antigens was controlled by immunofluorescence. VZV genome was detected by *in situ* hybridization using biotin-labeled VZV DNA fragment BamH1-C as a probe. Neurons were the only cell type infected in the cultures and viral genome was detected as early as 24 h. post infection. This system provides an *in vitro* model for the study of VZV maintenance and expression in human peripheral neurons.

This work was supported by the National Fund for Scientific Research (NSFR, Belgium).

OP18.20

EFFECTS OF ULTRAVIOLET IRRADIATION OF THE SKIN ON HERPES SIMPLEX VIRUS INFECTION OF MICE: ALTERATION IN IMMUNE FUNCTION MEDIATED BY EPIDERMAL CELLS AND IN THE COURSE OF INFECTION. T. Otani, R. Mori. Department of Virology, School of Medicine, Kyushu University, Fukuoka 812, Japan

Herpes simplex virus (HSV)-induced epidermal cell (EC)-mediated proliferative response of immune T cells was found to be impaired by pre-exposure of the skin to ultraviolet (UV) one to three days before preparation of EC in a dose-dependent fashion. This UV-induced impairment of the accessory cell function of EC was accompanied by a parallel reduction of the number of Ia^+ EC. These EC-stimulated immune T cells were then transferred to intracutaneously infected nude mice. Immune T cells stimulated with EC obtained from irradiated mice could not effectively clear HSV and caused development of zosteriform skin lesions, whereas normal-EC-stimulated immune T cells completely prevented the formation of zosteriform rash. In addition, mice irradiated with UV on shaved midflank skin two days before intracutaneous inoculation of HSV showed increased severity of infection and a higher incidence of latency compared with control mice.

These studies indicate that in vivo UV irradiation of the skin abrogate the immune function of EC both in vitro and in vivo, and affect the HSV pathogenesis.

OP18.21

COMPARISON OF VACCINE AND WILD STRAIN-INDUCED VARICELLA-ZOSTER VIRUS SPECIFIC ANTIBODY RESPONSE OF DIFFERENT SUBCLASSES OF IgG. Y. Asano, Y. Hiroishi, N. Itakura, S. Hirose. Departments of Pediatrics, Fujita Gakuen Health University School of Medicine, and Kariya Sogo Hospital, Aichi, Japan.

Commercially available mouse monoclonal antibodies to human IgG subclass (IgG1 to IgG4) had been applied to an enzyme-linked immunosorbent assay to measure IgG subclass specific antibodies to varicella-zoster virus (VZV) in children naturally infected with VZV and in varicella vaccine recipients. In children naturally infected with VZV, IgG1 antibody was detected 2 weeks after onset of the disease in all cases, its activity increased at 1 month after onset, and almost equal antibody value was maintained 10 years after infection. On the other hand low antibody activity was found in IgG2 only at 1 month of the disease. The highest antibody level of IgG3 was shown 2 weeks after onset of the disease, then it gradually decreased, and no antibody activity was detected 10 years later. IgG4 antibody was first detected 1 month after onset and almost equal level of antibody was shown 10 years after the disease. After inoculation of children with a live varicella vaccine, IgG subclass antibody responses to VZV were almost equal to those after natural infection. These results provide a evidence that the vaccine-induced humoral immunity is qualitatively equal with that after natural VZV infection.

OP18.22

ISOLATION OF CMV IN BLOOD. Evaluation with another diagnostic method. I. Calicó, G. Codina, L.Arcalís, J. Reina. Servicio de Microbiología. Universidad Autónoma de Barcelona. Ciudad Sanitaria "Vall d'Hebrón". Barcelona. Spain

340 Patients suspicious of infection by CMV have been studied by culture of the blood (leukocyte separation with Ficoll/Macrodex method) in human embryo fibroblast. At the same time urine, bronchoalveolar washings and biopsy especimens depending of the sintomatology of the patients were studied and whenever possible 2 samples of serum for anti-CMV antibodies deteccion were obtain (complement fixation test and Ig M anti-CMV by EIA. Serum from recently transfused patients were discarded.

115 Of the 340 patients showed to be infected by CMV in some of the tests : blood 48 positives / 115 patients, urine 74/99 , biopsies or bronchoalveolar washings 13/15, Ig M anti-CMV 44/96 , seroconversions 37/66 , high titers in C. F. test (= 1/256) 48/103.

In relative numbers the possitivities of blood cultures are clearly lower than in the other tests, except in patients with AIDS, since they had less demostrative serology.

The yield of the blood culture gets slighty better studying the patients near the onset of the febril syndrome.

OP18.23

ALTERED MEMBRANE ANCHOR AND CYTOPLASMIC DOMAINS OF HSV GLYCOPROTEIN gB-1 AFFECT ITS INTRACELLULAR SORTING. K.S. Raviprakash, L. Rasile, H.P. Ghosh. Department of Biochemistry, Faculty of Health Sciences, McMaster University, Hamilton, Canada.

Herpes simplex virus (HSV) buds from the inner nuclear membrane of infected cells. Unlike other HSV-1 glycoproteins, the glycoprotein gB-1 contains a 109 amino acid long cytoplasmic domain and a 69 amino acid long transmembrane domain which is predicted to make a triple pass through the membrane. In order to understand the roles of these two domains in gB-1 targeting, mutant gB-1 genes were constructed by site directed mutagenesis of cloned gB-1 gene. Mutant gB-1 proteins which contain only 6, 22, or 44 amino acids of the cytoplasmic domain are defective in their nuclear envelope localization. These mutant proteins, however, appear on the plasma membrane of transfected cells. Mutant gB-1 proteins that have partial deletion of the transmembrane domain, but contain intact cytoplasmic domain, on the contrary are localized on the nuclear envelope as is the wild-type gB-1. These mutant proteins, however, are slowly secreted out of the cell. Taken together, these results suggest that the long cytoplasmic domain may contain information for nuclear targeting of gB-1 and that the triple passing membrane anchor domain is perhaps necessary to stably anchor the protein which contains large amino terminal ectodomain and carboxy-terminal cytoplasmic domain. (Supported by MRC Canada and NCIC).

OP18.24

STRUCTURAL AND FUNCTIONAL ANALYSIS OF HERPES SIMPLEX VIRUS THYMIDINE KINASE. G. Palù, M.A. Biasiolo, S. Valisena, M. Palumbo. Institute of Microbiology and Department of Organic Chemistry, Faculties of Medicine and Sciences, University of Padova, Padova, Italy.

Although the nucleotide sequence of the thymidine kinase (TK) gene of herpes simplex virus type 1 and 2 (HSV-1,HSV-2) is being known, much is still to be understood on the structure-activity relationship of this enzyme. Important information could be gained at this level for a rationale design of new drugs. In an attempt to approach this problem we have expressed a few HSV-1 TK genes, some of which were sequenced by us, in a λ based vector.

The viral proteins were purified from E.coli and the enzyme activity was assayed with different nucleoside substrates. Kinetic parameters were shown to vary slightly in connection with minor modification of the aminoacid sequence occurring outside the supposed ATP-binding site. Purified enzymes were also studied by means of fluorescence spectroscopy and chiroptical techniques in the presence of the natural substrates and analogues thereof. In this regard the availability of dansyl-derivatives of thymidine was also exploited. Evidence is being seeked for the exact location within the enzyme of the nucleoside and ATP binding sites and definition of protein domains is attempted.

OP18.25

IN VITRO AND IN VIVO SENSITIVITY TO ACYCLOVIR OF HERPES SIMPLEX VIRUSES ISOLATED FROM JAPANESE WOMEN. M. Hashido[1], T. Kawana[2], Department of Maternal and Child Health[1], Department of Obstetrics & Gynecology[2], Faculty of Medicine, University of Tokyo, Tokyo, Japan.

Sixty-six herpes simplex virus isolates from genital herpes were examined in vitro using the plaque reduction assay to determine acyclovir sensitivity. The viruses consisted of 26 HSV-1 and 30 HSV-2 isolated from Japanese women, and 5 HSV-1 and 5 HSV-2 which were sent from U.S.A. including resistant strains to ACV. The sensitivity was expressed as an ID_m value. The ID_m values of acyclovir for HSV-1 in Japan ranged from 0.59 to 8.46 μM, with a mean ID_m of 2.92($\pm$1.85) μM. For HSV-2, the ID_m ranged from 3.80 to 12.05 μM, with a mean ID_m of 6.24($\pm$1.90) μM. These values were lower than those of ACV-sensitive strains in U.S.A. and it seemed that HSV in Japan were more sensitive to ACV.

In addition, our data indicated that in vitro sensitivity obtained by this study correlated with clinical efficacy of oral administration of ACV.

OP18.26

PHYLOGENETIC STUDIES OF HERPES SIMPLEX VIRUS TYPE 1 STRAINS ISOLATED IN JAPAN. H. Sakaoka, K. Sakino, K. Kurita, T. Aomori, T. Yamaguchi, and K. Fujinaga. Hokkaido University and Sapporo Medical College, Sapporo 060, Japan.

275 epidemiologically unrelated strains of herpes simplex virus type 1 (HSV-1) were compared by analysis of their genome structures using four restriction endonucleases, BamHI, KpnI, SalI, and HindIII. These isolates were obtained from seven areas in Japan between 1981 and 1985. To analyze the variation of HSV-1 isolates, clones of recombinant plasmid containing HSV-1 (F) BamHI fragments were used as probes. The frequencies of isolates having each cleavage pattern were determined by using programs of the KHSPSS system.

Using gains and losses of 19 sites, the total of 275 strains could be classified into 88 distinct cleavage patterns. However, the strains were clustered in four predominant cleavage patterns (6, 27, 60, and 77) which are genetically distant. In addition, there were highly significant difference in the proportion of some patterns between isolates from the areas of Japan. These results show that four major patterns of phylogenetically distinct HSV-1 strains have been perpetuated in the populations of Japan, and that clearly distinct HSV-1 strains have been distributed in those areas of Japan which are geographically apart from each other.

OP18.27

DETECTION OF CYTOMEGALOVIRUS WITH LABELED DNA PROBES.

C. Bourdon-Wouters, C. Sadzot-Delvaux[1], M.P. Merville-Louis, J. Piette[1] and B. Rentier[1]. Laboratory of Microbiology[1], University of Liège, Belgium.

Human Cytomegalovirus (HCMV) is a Herpesvirus which causes severe diseases and is able to persist for life in latent form. It can be reactivated especially in immunodepressed patients. The rapid detection of HCMV is essential prior to transplantation.

We have adapted genomic hybridization methods to the rapid, sensitive and specific detection of HCMV. The EcoR1-J fragment of the viral genome, ^{32}P or ^{35}S labeled by nick translation, was used as a probe for viral genome detection by dot blotting and *in situ* hybridization.

In situ hybridization allowed us to identify HCMV in cultures inoculated with human urine samples, and the sensitivity of the dot blotting method was such that we have been able to detect HCMV DNA directly in urine.

This work was supported by the National Fund for Scientific Research (NFSR, Belgium).

OP18.28

ACUTE AND LATENT HERPES SIMPLEX VIRUS INFECTIONS IN RATS.

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Herpes simplex virus (HSV) establishes a latent infection in sensory ganglia following either natural or experimental primary infection. The nature of the virus-cell interactions involved in the establishment and maintenance of latency remain unresolved. We have established a rat model for studying this phenomenon.

Sprague Dawley female rats of 70-100 g were inoculated with varying amounts of HSV type 1 at 4 different sites: ear pinna, rear foot pad, dorsal skin of rear foot, and sciatic nerve. Rats were sacrificed on day (d) 4 or 21 post-inoculation. Tissues at the site(s) of inoculation and corresponding sensory ganglia were assayed for infectious virus. At 4 d post-inoculation, 25-100% of inoculated tissues contained HSV; 33-100% of all rats were infected as evidenced by isolation of HSV from at least one ganglion; 33-100% of ganglia contained HSV. At 21 d, all injection site tissues were negative for HSV while 100% of rats (27.8-33.3% of ganglia) contained latent HSV as defined by recovery of HSV > 7 days after initiation of cocultivation. All control tissues and ganglia were negative. Rats may be a suitable model for examining factors that maintain and activate latent HSV in infected ganglia.

OP18.29

FUSION OF HERPES SIMPLEX VIRUS WITH LIVING CELLS AND LIPOSOMES, AS STUDIED BY FLUORESCENCE DEQUENCHING.

V. Citovsky,* A. Loyter,* E. Katz,** Department of Biological Chemistry, Institute of Life Sciences* and Department of Virology, Hadassah Medical School**, Hebrew University, Jerusalem, Israel.

Herpes simplex virus type 1 (HSV-1) was labeled with the fluorescent dye octadecylrhodamine-B-chloride. Fluorescent dequenching was observed following incubation of the virus with chick fibroblasts, BSC1 and vero cells both at pH 7.4 and 5.2. No increase in the extent of fluorescence (fluorescent dequenching) was observed following incubation with inactivated, non-infectious virions. Only fluorescent dequenching observed at pH 7.4, but not that at pH 5.2, was inhibited by lysosomotropic agents, such as methylamine and ammonium chloride, or by EDTA. Therefore, it is suggested that the fluorescent dequenching obtained at pH 5.2 is due to fusion with the cell plasma membrane, while that occurred at pH 7.4 resulted from fusion with membranes of intracellular organelles, following endocytosis of the virions.

Fusion of HSV-1 was also observed with liposomes composed of phosphatidyl-choline (PC) and phosphatidyl-ethanolamine (PE) or PC/PE/cholesterol. Only fusion with liposomes of the latter composition was shown to reflect the biological activity involved in the penetration of the virus into cells.

OP18.30

REGENERATION OF HUMAN SMOOTH MUSCLE CELL CULTURES FOLLOWING INFECTION WITH CYTOMEGALOVIRUS. J.J. Tumilowicz and J.J. Trentin. Division of Experimental Biology, Baylor College of Medicine, Houston, U.S.A.

Following infection with cytomegalovirus (CMV, strain AD169) human arterial smooth muscle cell (SMC) cultures were not completely destroyed but regenerated initially. A sub-population of small, phenotypically undifferentiated SMC appeared to be the predominant source of regeneration of the culture to approximately pre-infection levels under these experimental conditions. Some of these cells showed no evidence of CMV infection either by immunofluorescence, using anti-early CMV nuclear protein, or by a CMV gene probe technique. Regenerating cultures remained continuously susceptible, and regrowth was destroyed over a prolonged time as described previously (J. Virol. 56, 839, 1985). Viral output in these chronically infected SMC cultures rose and fell with cell number. Approximately three months following infection, a tiny fraction of the initial population number still persisted. A few of these cells reacted positively with anti-early CMV nuclear protein. The smallest of the undifferentiated SMC were now comparatively rare. The results suggest that regeneration can occur as long as a sub-population of small undifferentiated SMC exists. Some of these cells may not be susceptible to CMV until some phase of differentiation. Whether these are "phenotypically modulated" SMC (J. Cell Biol. 89, 379, 1981) or true blast cells is uncertain.

OP18.31

EPSTEIN-BARR VIRUS DNA BAM HI FRAGMENTS IN NASOPHARYNGEAL CARCINOMA TISSUES PASSAGED IN NUDE MICE. C.S.Yang,J.Y.Chen, Y.H.Ou, T.Y.Hsu,M.Y.Liu,T.S.Huang,R.Yang. Departments of Bacteriology and Pathology, College of Medicine, National Taiwan University, Taipei, Taiwan

DNAs extracted from nude-mouse-passaged NPC 295 and 306 at different passage levels were hybridized with 27 cloned EBV DNA Bam HI fragments obtained from B95-8 cells. During the serial passages in nude mice including one in vitro culture of NPC 295 and 306, two sarcomas each appeared. They were also included in this study. Anti-EBV DNase antibody in nude mice bearing the tumor was also tested.

The results were summarized as follows: 1. Except Bam HI fragments F,H,I,W and Y, the rest was found in both NPC DNAs in their proper size position, indicating that even after long passages these 22 Bam HI fragments existed stably in both carcinoma DNAs. 2. When Bam HI F was used as a probe, one additional small size band (2.0Kb) was found in all NPC 295 tissues but not in NPC 306. Co-cultivation of cultured epithelial cells from NPC 295 with cord blood leukocytes resulted in leukocyte transformation but NPC 306 cells did not. Therefore, the above finding is worth studying further. 3. In Bam HI fragments H and I, two larger bands were detected, suggesting that these two fragments are possibly integration sites of EBV DNA into NPC 295 and 306 DNAs. 4. EBV DNA Bam HI fragments were not found in sarcoma cells. 5. Anti-EBV DNase antibody was not detected in all nude mice bearing NPC 295 or 306 tumors.

OP18.32

O-GLYCOSYLATION OF HSV-1 GLYCOPROTEINS DEPENDENT ON HOST CELL SPECIFIED ENZYMES S. Olofsson, M. Lundström, S. Jeansson R. Datema*, Dept of Clinical Virology, University of Göteborg, Göteborg, Sweden and * Dept of Antiviral Therapy, Astra Läkemedel AB, Södertälje, Sweden

The herpes simplex virus (HSV-1) specified glycoprotein gC-1 contains a number of different O-linked oligosaccharides, distinguished by their different affinities for several lectins including HPA (main specificity terminal GalNAc) PNA (terminal Gal(β1-3)GalNAc) and WGA (for HSV glycoproteins, terminal sialic acid). We found that Mouse neuroblastoma (C1300) cells in contrast to other cell lines studied were able to provide gC-1 with HPA-binding oligosaccharides only. An examination of the glycosyl transferase pattern in C1300 cells indicated a general deficiency in galactosyl transferases while other enzymes, such as sialyl transferases were present in concentrations compared with those of other cell lines.

In conclusion, O-glycosylation of HSV-1 specified glycoproteins seems to be dependent largely if not totally, on host cell encoded glycosyl transferases.

OP18.33

PRODUCTION OF MONOCLONAL ANTIBODIES TO HUMAN CYTOMEGALOVIRUS (HCMV). EVALUATION OF DIFFERENT SCREENING METHODS. M. LANDQVIST, B. Zweygberg Wirgart and L. Grillner. Department of Clinical Microbiology, Karolinska hospital, Stockholm, Sweden.

Monoclonal HCMV antibodies were produced for studies on variation of strains and for diagnostic use. SP2/0 and F/0 myeloma cells were fused with spleen cells from Balb/c mice immunized with either a cell-lysate or a nuclear HCMV antigen. Hybrids were screened for reactivity to HCMV early (EA) and late (LA) antigens by indirect immunofluorescence (IIF). 70 were recognized as producing antibodies to HCMV, and so far, 14 have been cloned in limiting dilution. These monoclonal antibodies have been further characterized.

An ELISA using a HCMV antigen prepared from infected nuclei expressing immediate early (IEA), EA and LA was compared with IIF for detection of antibodies against these antigens. All monoclonal antibodies reacted against LA in ELISA although some very weakly. IIF against LA demonstrated a varying pattern of reactivity. Five also reacted with EA in both methods and three showed a reactivity against IEA. One of them showed a strong reactivity in ELISA as well as a distinct nuclear fluorescence against both EA and IEA. This monoclonal has been shown to be efficient in detecting EA in cell culture 24 h after inoculation of clinical samples.

The ELISA antigens were found to be useful for the screening of monoclonal antibodies against IEA, EA and LA. The reactivity of the monoclonal antibodies with different HCMV strains will be further studied.

OP18.34

T CELL PROLIFERATION IN RESPONSE TO RECOMBINANT gB-2 PEPTIDES. M.Tizard, W.L.Chan. Department of Microbiology, Medical School, Guy's Hospital, London, England.

It has been demonstrated that the 115K HSV-1 glycoprotein (gB-1) induces protective T cells in mice (Chan et al, J.Exp.Med. 162: 1304, 1985). To further investigate this observation protein sequences from the gB of HSV-2 (sharing > 85% homology at the amino acid level) were expressed as polypeptide fusions to Beta galatosidase. The location of specific polypeptide fusions was determined by dideoxynucleotide sequencing of the plasmid constructs encoding them. These proteins were purified by affinity chromatography prior to use in biological assay. Certain fusion proteins were able to stimulate proliferation in T cells, from the draining lymph nodes of mice primed with HSV-2 or HSV-1.

Data will be shown on the sub-types of T lymphocytes involved and lymphokines that they are stimulated to produce. The efficacy of these fusion proteins as protective immunogens, in mice, will be discussed.

OP18.35

THE NUCLEOTIDE SEQUENCE OF GLYCOPROTEIN B OF HERPES SIMPLEX VIRUS-TYPE 2 STRAIN 333: PREDICTED PRIMARY AND SECONDARY STRUCTURE. J. Zwaagstra and W.C. Leung. Depts. of Medicine, Medical Microbiology & Infectious Diseases, University of Alberta, Edmonton, Alberta T6G 2G3, Canada.

Glycoprotein B (gB) is one of the major viral-specified proteins of Herpes simplex virus (HSV). Its expression on the virion surface is required for viral infectivity. It is also a major neutralization antigen. The gB gene was isolated from a HSV strain 333 genomic library, and mapped by Southern and Northern blot analyses. DNA subfragments were cloned into M13 single stranded vectors and the entire sequence of both the coding and noncoding strands was determined and followed by computer analysis. Only one open translation reading frame was of sufficient size to encode the gB protein. The DNA sequence is 80% homologous with HSV-1 strains KOS and F and 95% homologous with HSV-2 strain HG52. The predicted length of gB2(333) is 902 amino acids. Hydropathic analysis of the protein predicts four major domains: a 22 amino acid signal sequence, a hydrophilic surface domain containing 8 N-linked glycosylation sites, a hydrophobic membrane spanning region and a hydrophilic cytoplasmic domain. Secondary structure analyses using the Chou and Fasman algorithm and prediction of antigenic regions by hydrophilicity, flexibility and accessibility algorithms indicated that gB2(333) exhibits both structural and antigenic differences from the other HSV strains.

OP18.36

MONOCLONAL ANTIBODIES FOR EARLY DETECTION OF CYTOMEGALOVIRUS (CMV). B. Zweygberg Wirgart, M. Landqvist, L. Grillner. Department of Clinical Microbiology, Section of Virology, Karolinska Hospital, S-104 01 Stockholm, Sweden.

We have compared two monoclonal antibodies for detection of early antigens of CMV in cell culture by immunofluorescence (IF). One, the CCH2 monoclonal antibody, was prepared in our laboratory, reacting with immediate early and early antigens in IF and ELISA and in Western Blot using a glycine extracted CMV antigen with a 43 000 dalton protein. The CCH2 antibody was compared with the commercially available monoclonal antibody 2H2.4 (Biotech Research Laboratories, Du Pont) directed against the 72 000 dalton early nuclear protein.

Human lungfibroblasts grown on coverslips in plastic plates were inoculated with 3-fold serial dilutions of the CMV Ad 169 strain. The cells were fixed 24 h after inoculation, and stained with the two monoclonal antibodies as primary antibodies. FITC-conjugated anti-mouse IgG (Cappel) was used as secondary antibody. Examination in a fluorescence microscope showed that the CCH2 and the 2H2.4 monoclonal antibodies detected CMV in equivalent dilutions of the CMV Ad 169 strain. Both monoclonal antibodies demonstrated a distinct staining of the nucleus in the CMV infected cells. The CCH2 monoclonal antibody was also equally efficient in detecting early antigens in urine specimens. Further evaluation will be done on clinical samples from kidney transplanted patients.

Monoclonal antibodies against early nuclear proteins are extremely useful for early diagnosis of CMV in cell culture.

OP18.37

ABROGATION OF T CELL CONTROL OF EBV LYMPHOPROLIFERATIVE POTENTIAL BY CYCLOSPORIN. L.J. York and L.F. Qualtiere, Immunovirology Group, Department of Microbiology, University of Saskatchewan, Saskatoon, Canada.

Primary infection with EBV results in the establishment of a carrier state in which the virus persists in a small population of B lymphocytes for the life of the host. The control of the lymphoproliferative potential of the virus is maintained, at least in part, by the reactivation of EBV-specific, MHC-restricted memory T cells.

Renal and cardiac allograft recepients undergoing immunosuppressive treatment with cyclosporin have been reported to have unusually high incidences of EBV genome-positive lymphoma. Human EBV-specific, MHC-restricted T cell clones were generated and maintained by repeated antigenic stimulation and addition of interleukin-2. These clones were used to determine cyclosporin's effect on the activation, proliferation and cytotoxic potential of individual EBV-specific cells and the possible abrogation of the cell's ability to inhibit outgrowth of infected B lymphocytes. Although cyclosporin does not inhibit the T cell's cytotoxic function, the reduction in membrane IL-2 receptor expression and hence proliferation limits the cell's ability to control EBV-infected B lymphocyte outgrowth.

OP18.38

USE OF LECTINS FOR DETECTION OF CLUSTERED O-LINKED OLIGOSACCHARIDES ON VIRAL GLYCOPROTEINS M. Lundström, S. Olofsson, S. Jeansson. Dept of Clinical Virology, University of Göteborg, Göteborg, Sweden.

The herpes simplex virus glycoprotein gC-1 contains pronase-resistant clusters of O-linked oligosaccharides, which can be isolated by lectin chromatography of pronase-digested glycoprotein.

We isolated one such glycopeptide binding to PNA and one binding to HPA and subjected both fractions to alkaline borohydride treatment to release O-linked carbohydrates. Both HPA and PNA-binding oligosaccharides were very small, and contained terminal GalNAc and Gal(β1-3)GalNAc respectively.

The lectins mentioned should be useful for detection of these types of O-linked carbohydrates also on glycoproteins of other viruses.

OP18.39

ACTION OF ALKALOIDS ISOLATED FROM AMARYLLIDACEAE ON HERPES-SIMPLEX VIRUS IN VITRO. J.Renard[1], T.Kim[1], Y.Imakura[2], M.Kihara[2] and S.Kobayashi[2]
[1]Department of Microbiology, Tokyo Medical College
[2]Department of Pharmacological Chemistry, Tokushima University, Tokushima, Japan.

Studies were carried out of the action of Amaryllidaceae Alkaloids and their derivatives on herpes simplex virus(type 1), the relationship between their structure and their antiviral activity and the mechanism of this activity.

All the Alkaloids used in these experiments are synthetized with N-benzylphenethylamine but those which could eventually prove to be antiviral agents have a ring of hexahydroindole with two OH radicals. It was shown that benzazepine compounds were neither cytotoxic nor antiviral while many structures containing dibenzazocine were toxic at low concentration.

It was established that the antiviral activity of Alkaloids occured because multiplication was inhibited, and not as a result of direct inactivation of extracellular viruses.

In the presence of the Alkaloids viral DNA polymerase activity decreased. Further experiments are to be conducted in order to elucidate the antiviral mechanism.

OP18.40

THE UNUSUAL MORPHOLOGY OF HUMAN CYTOMEGALOVIRUS (HCMV) ENVELOPES AND THE DISTRIBUTION OF gp-52. Linda M. Stannard, Janet Rider. Department of Medical Microbiology, University of Cape Town, South Africa, and PHLS, England.

Negative-staining electron microscopy was used to study the envelope morphology of released extracellular HCMV virions. They were frequently asymmetric with an accumulation of amorphous material on one side of the nucleo-capsid and a co-incidental protrusion of the envelope at right-angles to this. Envelope protrusions appeared as either bulbous or elongated tails. In-foldings of the envelope membrane sometimes resulted in the false impression of a double envelope. Monoclonal antibody to gp-52 (F5), coupled to colloidal gold, was used to identify that specific glycoprotein. Long slender spikes, similar in length to gB spikes of HSV, were labelled at their distal ends. They were most often present in homogeneous clusters, usually on the envelope protrusions, and seldom on the portion of the envelope overlying the bulge of dense material next to the nucleocapsid. Individual virions varied greatly from one another in the number of gp-52 spikes present.

Other morphologically distinctive glycoproteins, which were not labelled with gp-52 gold probes, were also observed. Dense bodies were also strongly labelled with gp-52 probes.

OP18.41

PREVALENCE RATES OF NEUTRALIZING ANTIBODIES TO HERPES SIMPLEX VIRUS (HSV) TYPE 1 AND 2. Y. KAZUYAMA, F. TAGUCHI. R & D Center of Hygienic Sciences and Department of Microbiology, Kitasato University School of Hygienic Sciences, Tokyo, Japan.

A total of 1400 serum samples from various age groups of healthy adult donors were examined by the complement fixation (CF) test for the prevalence rate of CF antibodies and also by the neutralization (NT) test for type specific NT antibodies to HSV type 1 (HSV-1) and HSV type 2 (HSV-2). The NT antibody prevalence in 1400 sera tested was 67% and 18% to HSV-1 and HSV-2, respectively. The frequency of NT antibodies to both HSV-1 and HSV-2 increased gradually as the age of groups became higher. The antibody positive rates for both HSV-1 and HSV-2 were significantly higher in female than in male. Of serum samples in elder age groups 6 to 10% sera were NT antibody positive but CF antibody negative, although serum antibody titers determined by CF and NT tests were well correlated each other in younger age groups.

OP18.42

LOCALIZATION OF A VARIANT OF HERPES SIMPLEX VIRUS TYPE 1 AND THE CHARACTERISTIC DNA STRUCTURE. H. Eda[1], S. Ozawa[2], K. Yoshino[2], K. Hayashi[3], K. Yanagi[1]. 1)Department of Virology and Rickettsiology, National Institute of Health, Tokyo, 2)Yamanashi Institute of Health and Pollution Yamanashi, 3)Kohriyama Institute of Medical Immunology, Kohriyama, Japan.

We analyzed more than 550 fresh isolates of herpes simplex virus type 1 (HSV-1) from more than twelve regions in Japan by DNA finger printing with restriction endonucleases. We found a particular variant which has a set of mutations around 3.7, 16.5, 78.1, and between 69 and 74 (map unit) on the variant's genome by Southern's blot hybridization. It was found that proportions of the variant of the total isolates analyzed in four East regions in Japan were 0, 11, 5, and 7% on one hand, and those in five West regions in the country was 61, 47, 32, 50, and 27% on the other hand. The percentages in three central regions in Japan were 17, 16, and 22%. Thus, our results clearly showed that DNA structure of HSV isolates has some geographical characteristics. They seem to be useful to discuss a mode of divergence of HSV genome and/or transmission in relatively well conserved local communities on historically isolated Japanese Islands.

OP18.43

PROTECTION OF MICE AGAINST HERPES SIMPLEX VIRUS TYPES 1 AND 2 INFECTION USING GLYCOPROTEINS gB-1 OR gC-1. P.L. Roberts. Department of Microbiology, University of Leicester, Leicester, U.K.

HSV-1 glycoproteins gB-1 and gC-1 were purified from infected cells by immunoadsorption chromatography using monoclonal antibodies. Mice immunized with gB-1 contained high levels (1/400-1/800) of neutralizing antibody active against both HSV-1 and -2. Glycoprotein gC-1 also induced similar high levels of neutralizing antibody against HSV-1, but only very low levels (1/10) against HSV-2.

Mice immunized with gB-1 or gC-1 were protected against intracerebral challenge (ic) with a dose of the homologous HSV-1 strain HFEM (30 LD-50) which normally produces encephalitis and death. The mice were also protected against footpad challenge with a low-passage human HSV-1 isolate which normally produces a severe bilateral hind-leg paralysis.

The ability of the HSV-1 glycoproteins to cross-protect against HSV-2 was also investigated. Mice that received gC-1 or gB-1 were protected against ic challenge with the HSV-2 laboratory strain 3345. Interestingly, however, 66% of these surviving mice developed cataracts 3 months later. This effect was also found in control mice that were challenged with only 1 LD-50 of virus. Immunized mice were also protected against footpad challenge with a low-passage human HSV-2 isolate.

OP18.44

CLONING AND EXPRESSION OF HERPES SIMPLEX VIRUS GLYCOPROTEINS IN E. COLI. A.C. Fluit, J.A.G. van Strijp, C.P.M. van Kessel and J. Verhoef. Laboratory for Microbiology, State University of Utrecht, Catharijnesingel 59, 3511 GG Utrecht, The Netherlands.

In host defence against Herpes Simplex Virus (HSV) infections the virus coded glycoproteins appear to be the most important antigens. Especially interesting is glycoprotein C (gC) of HSV type 1, which can function as a receptor of C3b an activated component of the complement system. We have isolated the coding sequences of the HSV type 1 glycoproteins gB and gC. These sequences are placed behind the λpR promotor under control of the thermosensitive λcI857 repressor. Transformants are screened for expression of the cloned proteins. First indications are that both proteins are expressed.

OP18.45

REGULATION OF EXPRESSION OF HUMAN CYTOMEGALOVIRUS GENES IN HUMAN FIBROBLASTS. John Sinclair. MRC Clinical Immunology Research Group, Royal Postgraduate Medical School, London UK.

Human cytomegalovirus (HCMV) shows a strict host cell type specificity with high titres of virus only produced in human diploid fibroblasts. Undifferentiated human teratocarcinoma cells are also non-permissive for HCMV infection but differentiation of the teratocarcinoma cells with retinoic acid leads to a reversal of this block and full permissive infection. Consequently, it is likely that, as well as viral factors, host cell factors play a fundamental role in the control of HCMV gene expression. As levels of cellular oncogene (c-onc) expression are known to be correlated with cell proliferation and differentiation and c-onc genes may play a role intrans-regulating other cellular genes, experiments have been carried out to determine whether changes in c-onc gene expression affect HCMV gene expression. By co-transfection, pEsCat (an expression vector based on the HCMV major immediate early gene) has been introduced into cells and its level of expression assayed in the presence or absence of the myc and fos oncogenes placed under the control of constitutive or inducible promoters. Results show that changes in levels of onc gene expression do affect the expression of pEsCat, suggesting that levels of c-onc gene expression may directly or indirectly trans-regulate HCMV gene expression. This can be correlated to changes in c-onc gene expression during viral infection.

OP18.46

REGULATION BY RECOMBINANT IL-2 OF PROTECTIVE IMMUNITY AGAINST RECURRENT HSV-2 GENITAL INFECTION IN GUINEA PIGS. Adriana Weinberg, Michael Konrad, and Thomas C. Merigan. Division of Infectious Diseases, Stanford University, Stanford, California.

The goal of our study was to determine if rIL-2 could modify the recurrence pattern of chronic HSV-2 genital infection in guinea pigs. Animals chronically infected were submitted to the following daily doses of rIL-2 for 4 weeks: 5×10^3 u, 5×10^4, 2.5×10^5, placebo. We observed a significant decrease of the incidence of new recurrent lesions with 5×10^4 (rate of recurrence 0.08 vs. 0.21 in controls), whereas the other rIL-2 regimens did not affect the overall recurrence rate. However, 5×10^4 was effective only during the first 3 weeks of use and 2.5×10^5 markedly decreased the rate of recurrence in the first week of treatment but not in subsequent weeks. The loss of clinical protection in both groups coincided with the production of neutralizing antibodies to rIL-2. Of the immune mechanisms possibly involved in the protective effect of rIL-2 in chronic HSV-2 disease production of γ IFN correlated well with clinical protection and it dropped when neutralizing antibodies to rIL-2 developed. NK and LAK activity were also increased in the treated guinea pigs. Antibody titers and lymphocyte proliferation to HSV antigen were similar in rIL-2 and placebo recipients.Finally, we found that the rIL-2 immune stimulation was equally protective against recurrent HSV-2 disease as the viral suppression of acyclovir. However, the biological activity of both drugs was not additive when they were co-administered.

OP18.47

ESTABLISHMENT AND CHARACTERIZATION OF VARICELLA-ZOSTER VIRUS PERSISTENCE IN VERO CELLS. K. Konno, T. Yokota, S. Shigeta. Department of Bacteriology, Fukushima Medical College, Fukushima City, Japan.

A persistent varicella-zoster virus(VZV) infection was established in Vero cells (African green monkey kidney cells) and has been maintained with dynamic-state for over 2 years after initial infection with cell-free VZV of CaQu strain. The VZV-infected Vero cells grew as well as uninfected cells, except during occasional periods of crisis (increased viral CPE). Cells could readily survive the crisis when they were maintained for twice the usual time interval (14 rather than 7 days) before subculture. Immunofluorescence and infectious center assays were performed periodically at the time of cell-passage to examine the extent of infection in the cultures; the mean level of VZV antigen-positive cells was 22% (range, 8 to 45%), and infectious center averaged 6.6% (range, 3 to 17%). VZV recovered from the culture had increased cyncytia-forming activity as compared with the parental VZV. Forty-two clones derived from the VZV-infected cell line produced neither infectious cell nor VZV antigen-positive cell. VZV-DNA sequence was not found in any of these clones by spot hybridization with SstI G and H fragments of VZV-DNA. Susceptibility of these clones to the parental and the recovered VZV was similar to that of uninfected Vero cells. Interferon was not detectable in the cultures.

OP18.48

IDENTIFICATION OF VARICELLA ZOSTER VIRUS GENE PRODUCTS USING ANTI-PEPTIDE ANTIBODIES AND VACCINIA RECOMBINANTS. Paul R. Kinchington, W. T. Ruyechan, J. H. Subak-Sharpe, and J. Hay. Depts. of Microbiology and Biochemistry, Uniformed Services University of The Health Sciences, Bethesda, MD USA and Institute of Virology, Glasgow, Scotland.

We have used two approaches to further characterize several gene products of varicella zoster virus (VZV).

Peptides corresponding to parts of open reading frames within the VZV genome have been synthesized and used to generate anti-peptide antibodies. We have identified proteins coded for by VZV genes equivalent to the herpes simplex type one major DNA binding protein, the immediate-early polypeptide ICP4, the virion trans-activating protein and a putative virion protein kinase. Studies with the antibodies directed against the VZV major DNA binding protein show that it is located almost entirely within the nucleus of infected cells and has a strong binding affinity for single stranded DNA.

We have also constructed vaccinia virus recombinants expressing VZV glycoproteins gpI and gpV. These are being used to determine the role of these glycoproteins as targets for virus neutralizing antibodies and cytotoxic T lymphocytes.

OP18.49

A COMPARISON OF COMPLEMENT FIXATION AND WESTERN BLOTTING REACTIVITY IN NON-ACUTE SERA FOR THREE HERPESVIRUSES.
D.R.Harper, K.M.Liu, H.O.Kangro. Department of Virology, Medical College of St. Bartholomew's Hospital, London, United Kingdom.

A comparison was undertaken of the reactivity shown with sera from 36 healthy blood donors to cytomegalovirus (CMV), herpes simplex virus type 1 (HSV), and varicella-zoster virus (VZV). Lysates of infected MRC5 cells were used as immunoblotting antigen, which was reacted with serum then with ^{125}I-anti IgG prior to autoradiography. Complement fixation (CF) testing was performed at 15°C.

Sera showed CF titres from <4 to 256 for all three viruses, and exhibited a wide variation in western blot reactivity. In all, four negative results were obtained by western blotting for sera which were positive by CF. Correlation between the results obtained with the two methods was relatively poor for both CMV and VZV ($r=0.3$, $p\approx0.05$), but HSV showed a much stronger correlation ($r=0.8$, $p<<0.001$). This was evident whether western blot reactivity was quantitated by number of bands or by intensity, which two parameters showed extremely high correlation ($p<0.001$) for all three viruses. While CF titres were broadly comparable for all three viruses, western blot reactivity varied more widely, and was much lower for VZV than for HSV or CMV. It is clear that care is required when interpreting results obtained for Herpesviruses by western blotting.

OP18.50

Enzygnost® Anti EBV: An ELISA for the detection of human IgM, IgG and IgA antibodies against Epstein-Barr Virus

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Research Laboratories of Behringwerke AG, Marburg, FRG.
Epstein-Barr Virus (EBV) infections are well described as causing or accompanying severe diseases in man, such as infectious mononucleosis (IM), Burkitt's Lymphoma, nasopharyngeal carcinoma, congenital or acquired immuno deficiencies. In an indirect ELISA of the heterogeneous type EBV-antigens, derived from TPA-induced lymphoblastoid cell-line B95-9, are bound to the solid phase of microtitration plates. EBV-specific antibodies in serum samples are detected by an anti-human IgM-, IgG- or IgA antibody/POD-conjugate. Unspecific binding is visualized by an appropriate control antigen prepared from the same cell blocked for EBV-expression. For ELISA evaluation we tested a panel of approximately 300 pos. and neg. sera. Cut off values for pos./neg. discrimination of IgM, IgG and IgA antibodies were verified by 100 negative sera.
In a first study we found IgA antibody as a valuable marker for an IM and an antibody pattern for other EBV-related diseases as already published. From the preliminary results we conclude, that the specificity and objectivity of evaluation of the Enzygnost® Anti EBV could be a suitable alternative to the generally used heterophile antibody testing and indirect immunofluorescence technique.

OP18.51

STRUCTURE AND COMPLETE NUCLEOTIDE SEQUENCE OF MAREK'S DISEASE HERPESVIRUS A ANTIGEN GENE. P.M. Coussens and L.F. Velicer, Department of Microbiology and Public Health, Michigan State University, East Lansing, Michigan 48824, USA.

Marek's Disease Herpesvirus A antigen (MDHV-A) is a secretory glycoprotein with an apparent molecular weight of 55-67, as determined by SDS-PAGE. The gene encoding MDHV-A has been subcloned from GA strain MDHV (R. Isfort et al., submitted) and its complete nucleotide sequence determined. S1 nuclease protection analyses were used to map the putative 5' transcription initiation site to approximately 100 bp 3' of the unique Pvu II site in the MDHV Bam HI B fragment. Nucleotide sequence analysis of the Pvu II-EcoRI fragment (2.35 kb) revealed a single open reading frame of 1515 bp. Translation of this open reading frame would produce a polypeptide of 505 amino acids. The predicted MDHV-A polypeptide sequence contains 8 potential N-linked glycosylation sites (Asn-x-Ser/Thr) and an amino-terminal hydrophobic domain. The absence of a carboxy-terminal transmembrane anchor sequence may explain the secretory nature of the MDHV-A polypeptide. Homologies of MDHV-A to other herpesvirus glycoprotein genes, including the immunologically related herpesvirus of turkeys (HVT) A antigen gene, and initial expression studies will be discussed.

OP18.52

SYNTHESIS AND PROCESSING OF THE MAREK'S DISEASE HERPESVIRUS B ANTIGEN GLYCOPROTEIN COMPLEX. I. Sithole, L. F. Velicer. Department of Microbiology and Public Health, Michigan State University, East Lansing, Michigan 48824, USA.

Marek's Disease Herpesvirus B-antigen (MDHV-B) consists of glycoproteins gp100, gp60, and gp49, as determined by immunoprecipitation with anti-MDHV-B followed by SDS-PAGE. Use of tunicamycin, an inhibitor of N-linked glycosylation, revealed two putative precursor polypeptides of 88kd (pr88) and 44kd (pr44). Pulse-chase studies show gp100 is cleaved to gp60 and gp49. The ionophore monensin inhibited this proteolytic cleavage, with accumulation of gp100. Endo-F, which cleaves N-linked oligosaccharides of both high mannose and complex types, cleaved gp100 to pr88, indicating pr88 is an intermediate in the biosynthesis of MDHV-B. Endo-H, which cleaves N-linked oligosaccharides of the high mannose type, cleaved gp100 to pr88, and cleaved both gp60 and gp49 to pr44. Four classes of protease inhibitors had no effect on appearance of pr44, suggesting it is not an artifact of proteolytic degradation of pr88. However preliminary partial proteolytic cleavage data of pr88 and pr44 suggests that they are related. Therefore pr88 is glycosylated to gp100 which in turn is cleaved to gp60 and gp49. While the role of pr44 as a precursor remains to be elucidated more rigorously, it appears to be a component of both gp49 and gp60. Thus it is tempting to speculate that the initial processing step involves formation of pr88 as a dimer of two pr44 molecules.

OP18.53

ANALYSIS OF THE CODING CAPACITY OF THE GENETIC INFORMATION LOCATED BETWEEN 0.762 TO 0.789 MAP UNITS OF THE HSV-1 GENOME AND ITS INFLUENCE ON THE VIRAL PATHOGENICITY. A.Rösen-Wolff, W.Lamadé, Y.Becker*, G.Darai. Institute of Medical Virology of the University of Heidelberg, FRG; *Department of Molecular Virology, Faculty of Medicine, The Hebrew University, Jerusalem, Israel.

The HSV-1 strain HFEM is apathogenic for the tree shrew and mice by the intraperitoneal (IP) route due to a deletion in the genome coordinates 0.762-0.789. Insertion of the BamHI DNA fragment B (0.738-0.809 mu)or of the MluI DNA fragment (coordinates 0.7615-0.789) molecularly cloned from the virulent HSV-1 strain F restored the IP pathogenicity to strain HFEM. Stable intratypic recombinant virus was established, virulent for both animals. The determination of the nucleotide sequence flanking the deletion in the genome of the avirulent HSV-1 HFEM and the corresponding region of the genome of the virulent recombinant virus RH1C1 was carried out and compared to the DNA sequences of the virulent wild type HSV-1. This analysis revealed the presence of different ORFs in this region. The functional activity of the hypothetic genes is still unknown and the subject of further investigation.

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OP18.54

ANTIBODY TITERS TO EPSTEIN-BARR VIRUS DETERMINED NUCLEAR ANTIGENS 1, 2, AND 3 IN JAPANESE PATIENTS' SERA. N. Inouye, S. Hongoh Harada, A. Oya, K. Yanagi, Department of Virology and Rickettsiology, National Institute of Health, Tokyo, Japan.

It was reported that many sera from patients with rheumatoid arthritis reacted with Epstein-Barr virus determined nuclear antigens (EBNAs) 2 and 3. Examination of antibody titers to each EBNA, i.e., EBNA 1, 2, 3 etc. in human sera may be useful to serodiagnosis in EBV associated diseases.

We have constructed recombinant lambda phages by inserting parts of coding regions of EBNAs 1, 2, and 3 into lac Z gene on λ gt11 DNA. The β-galactosidase-EBNA fusion protein molecules were synthesized in E. coli. We also established COS-1 and other cell lines which stably produce EBNA-1 by transfection of recombinant DNA which we constructed using BamHI K fragment of B95-8 DNA from Dr. E. Kieff and pSV2gpt DNA. Those fusion proteins and EBNA polypeptides of authentic size synthesized in animal cells were used as antigens for immunoblotting and/or immunofluorescence tests. Sera of Japanese patients (Tokyo) diagnosed as having rheumatoid arthritis gave results similar to a report from Australia (Sculley et al.) etc.

OP19.1

TWO DIFFERENT SATELLITE RNAs ASSOCIATED WITH CHICORY YELLOW MOTTLE VIRUS. P. Piazzolla[+], L. Rubino[+], M.E. Tousignant, J.M. Kaper. [+]Dipartimento di Patologia Vegetale, Università degli Studi di Bari, and Centro per lo studio dei virus e delle virosi delle colture mediterranee, Bari, Italy. Plant Protection Institute, ARS, USDA, Beltsville, MD 20705, USA.

Chicory yellow mottle virus (CYMV) is a nepovirus isolated from chicory in Southern Italy of which three strains were described, named CYMV-T, CYMV-C and CYMV-RS.

CYMV-T (type strain), besides the genomic RNAs (2.3 and 2.0 x 10^6 daltons respectively), has two additional RNA species: (i) a RNA with mol. wt. of c. 500,000 (500K); (ii) a smaller RNA with mol. wt. of c. 170,000 (170K) which may occur in multimeric forms. The 170K RNA was previously shown to be a satellite RNA. CYMV-RS (ringspot strain) is satellite-free in nature. CYMV-C has the 500K RNA only.

Northern blot hybridization experiments showed that the 500K RNA does not hybridize with cDNAs to the 170K RNA or CYMV-T and CYMV-C genomic RNAs, indicating that this RNA is a satellite. Thus, it appears that CYMV-T can support two different satellite RNAs.

OP19.2

CHARACTERIZATION OF A SATELLITE RNA ASSOCIATED WITH A LUTEOVIRUS. W.A. Miller, T. Hercus, P.M. Waterhouse, W.L. Gerlach. CSIRO Division of Plant Industry, Canberra, Australia.

A satellite RNA has been found associated with the RPV serotype of barley yellow dwarf virus. This RNA has properties consistent with those of a satellite: (*i*) it is small (321 nucleotides) and occurs in high levels in purified virus preparations, (*ii*) it has no homology with host plant or viral genomes, (*iii*) linear, circular, and multimeric forms have been detected, (*iv*) multimers of the RNA self-cleave into monomers *in vitro*, (*v*) the complete nucleotide sequence has been determined, revealing a region showing primary and secondary structural similarity with the self-cleaving sites of satellites of sobemo- and nepoviruses, (*vi*) the RNA is not required for infection; field isolates have been found which lack the satellite. The satellite RNA appears to be RPV-specific: it was not maintained by a preparation of the PAV serotype which was isolated from a mixed infection of the PAV and RPV serotypes containing the satellite. Experiments are underway to determine if this satellite RNA affects disease symptoms. This is the first report of a small satellite RNA of a luteovirus, extending the known small number of plant virus groups capable of supporting satellite RNAs.

OP19.3

DIFFERENCES IN GROWTH PROPERTIES OF HEALTHY AND VIROID-CONTAINING CELLS IN CULTURE. N. Duran-Vila and *J.S. Semancik. Instituto Valenciano de Investigaciones Agrarias, Moncada (Valencia), Spain. *Department of Plant Pathology and Cell Interaction Group, University of California, Riverside, California 92521, USA.

Callus and cell suspension cultures infected with the citrus exocortis viroid (CEV) display no apparent symptoms in gross morphology or growth rates under the standard conditions of culture maintenance. However differences in growth characteristics can be exposed by modification of the culture medium and variation of temperature regimes. Supplementation of culture media with D-xylose, D-arabinose or L-arabinose, alone or in combination with auxin induces marked differences in growth response which can be utilized to define a selective medium to recover infected cells from mixed cell cultures. Conversely, the addition of hydroxyproline enhances the selective recovery of healthy cells from a mixture of healthy and CEV-containing cells.

The possible differences in carbohydrate uptake, utilization and storage will be discussed as well as the role of temperature and other standard components of the culture medium as mechanisms to expose differences between healthy and viroid infected cells in culture.

OP19.4

PROPERTIES OF AN IN VITRO VIROID-SYNTHESIZING COMPLEX FROM NUCLEAR EXTRACTS. M. F. RIVERA-BUSTAMANTE and J. S. SEMANCIK, Plant Pathology Department, University of California, Riverside, Riverside, CA 92521 USA.

A viroid RNA synthesizing complex was solubilized from nuclear fractions of citrus exocortis viroid (CEV)-infected Gynura aurantiaca leaf tissue employing the detergent sarkosyl. The complex displayed characteristics of a DNA-dependent RNA polymerase II-like enzyme acting on a deproteinized template with optimal requirements of 1 mM $MnCl_2$, 10 mM $MgCl_2$, 25 mM $(NH_4)_2SO_4$. The complex also demonstrated a zinc dependency and 90% inhibition with alpha-amanitin (4 µg/ml).

Viroid synthesizing activity was inhibited by RNAse and proteinase but not affected by DNAse I or actinomycin D (20 µg/ml).

Analysis by sucrose gradient sedimentation resulted in the separation of two subpopulation of viroid molecules: nascent (labeled) CEV associated with the faster sedimenting complex, while the bulk of the native CEV population sedimented at a lighter density than the complex.

The predominantly circular characteristic of the nascent molecules found associated to the complex suggests that they are a primary form of the early product of CEV synthesis. With these findings a modification of the currently accepted rolling circle model for viroid replication can be proposed.

OP19.5

INTERACTION OF CMV-SATELLITE RNAs WITH RNAs OF HELPER AND NON-HELPER VIRUSES. F. García-Arenal and P. Palukaitis, Dept. of Plant Pathology, E.T.S.I. Agronomos, Madrid, Spain and Dept. of Plant Pathology, Cornell University, Ithaca, NY, USA.

The satellite RNA of the Q-strain of cucumber mosaic virus (CMV) has been shown to anneal to sequences contained within the coat protein gene of Q-CMV; i.e., to RNA 3 and RNA 4. In addition it has been suggested that this interaction could play a role in the sat RNA regulation of viral functions (Rezaian and Symons, 1986. Nucleic Acids Res. 14, 3229). We have analyzed the sequences of six sat RNAs of CMV differing in their pathogenicity, to see how general this interaction could be. When these sat RNAs were annealed to the RNAs of several different helper viruses (i.e., both CMV strains and tomato asperny virus), such interactions were indeed observed to occur. However, the nature of the interaction varied with different combinations of sat RNA and helper virus RNAs. In addition, with some sat RNAs annealing was shown to occur with RNA 2 as well as with RNAs 3 and 4. Furthermore, some sat RNAs also annealed to RNAs of the non-helper viruses, brome mosaic virus, cowpea mosaic virus and tobacco mosaic virus. The implications of such interactions will be discussed.

OP19.6

BIOLOGICAL ACTIVITY OF T7 TRANSCRIPTS OF TWO CLONES OF A SATELLITE RNA OF CUCUMBER MOSAIC VIRUS. G. Kurath and P. Palukaitis. Dept. of Plant Pathology, Cornell University, Ithaca, NY, USA.

Molecular cloning of the (D)CARNA 5 (previously known as n-CARNA 5) necrosis inducing satellite RNA of cucumber mosaic virus produced a prototype clone and a sequence variant clone. The sequence variant contained ten nucleotide changes between positions 70-160 which rendered that region identical to the corresponding region of a satellite RNA which does not induce necrosis. T7 RNA polymerase transcripts of each clone replicated in both tobacco and tomato, and the progeny satellite RNAs did not retain the 54 nucleotide non-satellite sequence at the 5' end of the T7 transcripts. RNase T1 fingerprint analysis of both T7 transcripts and progeny satellite RNAs proved that each transcript was faithfully replicated in tobacco. Replication of either transcript in tomato resulted in lethal necrosis, suggesting that the determinant of necrosis induction lies outside the region between nucleotides 70 and 160. We are using these clones to investigate satellite RNA functional domains and population heterogeneity.

OP19.7

HETEROGENEITY AMONG SATELLITE RNAs ASSOCIATED WITH CUCUMBER MOSAIC VIRUS ISOLATED IN JAPAN. K. Hanada. Department of Plant Protection, National Agriculture Research Center, Tsukuba, Japan.

Three distinct Satellite RNAs (Sats OY, Y and E) were found in Japan, and their complete nucleotide sequences were determined already (S. Hidaka et al. 1984). Newly four distinct Sats (Sats Cu, Ka, F2 and Mt) were detected in cucumber mosaic virus which did not cause necrosis on tomato. When the mobility of each Sat was compared by gel electrophoresis under semi-denaturing conditions, all Sats showed characteristic mobility in the gel; OY, Cu, Ka, Y, F2, E and Mt (from the slowest). Recently Sat Tn was isolated from tomato bearing systemic necrosis in the fields and this Sat was the causal agent of tomato necrosis. These eight Sats were distinct from the two Sats found in Japan reported by K. Yoshida et al. (1985).

OP19.8

CHARACTERISTICS OF SATELLITE TOBACCO RINGSPOT VIRUS COMPLEMENTARY RNA FROM INFECTED TISSUE. B.K. Gregory, J.M. Buzayan and George Bruening, Department of Plant Pathology, University of California, Davis, California 95616, U.S.A.

Satellite of tobacco ringspot virus RNA (STobRV RNA) shows no extensive homology to TobRV genomic RNA sequences and is propagated only in the presence of TobRV. The satellite RNA acts as a parasite of TobRV, decreasing virus accumulation and the severity of induced symptoms. STobRV can represent 90% of the RNA found in virions. The abundant encapsidated "monomeric" form of STobRV RNA has 359 nucleotide residues (nt) and is arbitrarily designated as having the (+) polarity. Lesser amounts of dimeric and higher order multimeric forms also are encapsidated, and monomers and multimers of both polarities are present in infected tissue, as are circular forms. In vitro transcription of cDNA clones of a circularly permuted STobRV RNA sequence generated autolytically processing RNAs, the sites of autolysis for (+) and (-) RNAs residing 48 nt apart in the STobRV RNA sequence (J.M. Buzayan, W.L. Gerlach and G. Bruening, Nature 223:349-353 (1986)). These results support a rolling circle scheme for STobRV RNA replication. We have developed conditions that resolve (-) and (+) STobRV RNA and have analyzed STobRV (-)RNA from infected tissue for terminal residues and nucleotide sequences to determine whether it might be generated by an autolytic reaction like that observed in vitro.

OP19.9

STUDIES ON CELLULAR VIROID-PROTEIN COMPLEXES

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The location in the nucleus and the cellular complexes of viroids and its replicative intermediates were studied by fractionation techniques and crosslinking after UV-irradiation. A nucleoli fraction was prepared from green leaf tissue of viroid-infected tomato plants. The nucleoli were digested by DNase and fractionated in 10S-particles and larger components. Viroids and its replicative intermediates have been analysed by return gel electrophoresis and hybridazation using RNA probes. Circular viroids have been detected in the 10S-particles from nucleoli. Besides viroids these particles consist of several proteins which were analyzed by gel electrohoresis. The same viroid-protein complexes could be identified after crosslinking viroids with its contacting proteins by UV-irradiation in intact nuclei. A very similar location of viroids in nuclei was found if viroid strains of different pathogenicity were compared. The studies on viroid-protein complexes were extended to replicative intermediates and its complexes with the RNA polymerase II.

OP19.10

CONTROL OF CHERRY LEAF ROLL VIRUS BY A SATELLITE RNA THAT IT FAILS TO SUPPORT. F. Pons, A. Rowhani, G. Bruening. Department of Plant Pathology, University of California, Davis, USA.

Cherry Leaf Roll Virus (CLRV) is a member of the nepovirus group of plant viruses, and the causal agent of the black line disease of English walnut trees. Tobacco Ringspot Virus (TobRV) is the type member of the same group. TobRV supports the replication and encapsidation of a small (359 nucleotides) RNA molecule named Satellite RNA of TobRV (STobRV RNA) that essentially acts like a parasite of TobRV, reducing drastically the symptoms caused by it. We have studied, both in vivo and in vitro the effects of STobRV on CLRV. The main conclusions are: i) CLRV is unable to support the encapsidation of STobRV RNA, ii) STobRV RNA interferes in vivo with the replication cycle of CLRV when co-inoculated on cowpea leaves, iii) STobRV interferes in vitro with the level of translation and processing of CLRV polyproteins. The results will be presented in the context of a possible way of control of the black line disease of walnut trees.

OP19.11

SYMPTOM EXPRESSION, VIRUS ACCUMULATION AND RNA SYNTHESIS OF A VIRULENT CUCUMBER MOSAIC VIRUS(CMV) IN PLANT PREINOCULATED BY CMV WITH OR WITHOUT SATELLITE RNA. G-S. Wu, L-Y. Kang, P. Tien. Department of Virology, Institute of Microbiology, Academia Sinica, Beijing, People's Republic of China.

Tobacco(Xanthi nc.) and pepper plants were preinoculated by Chinese CMV (with R-CARNA 5 or without R-CARNA 5) or CMV-S then challenged by a virulent CMV devoid o. CARNA 5. After challenge the host symptom expression, virus accumulation and RNA synthesis were investigated by symptom survey, protein A sandwich Elisa and incorporation of ^{3}H-uridine in viral and satellite RNA.

The plants preinoculated by CMV with CARNA 5 showed very mild symptoms, low virus concentration and high incorporation of ^{3}H-uridine in double-stranded CARNA 5 but low incorporation in viral genomic single-stranded(ss) and double-stranded (ds) RNA, in particular showed a sharp increase of ^{3}H incorporation in CARNA 5 after challenge. The plants preinoculated by CMV without CARNA 5 showed severe symptoms, high virus concentration and high incorporation of ^{3}H-uridine in viral genomic ss- and ds-RNA. These results revealed the protection of CARNA 5 containing CMV agent is different from conventional cross-protection effects among different CMV. In the plants the latter was superposed, or supplanted by the former.

OP19.12

PEAR RUSTY SKIN DISEASE——— A VIROID DISEASE. W. Chen, X-C. Yang, P. Tien. Department of Virology, Institute of Microbiology, Academia Sinica, Beijing, People's Republic of China.

Pear rusty skin symptoms were observed in Beijing, Shanxi province and Hebei province in China. Nucleic acids were extracted from diseased tree barks as well as healthy ones. In the extracts from diseased tree barks, one or two low molecular weight circular RNA were detectable as showed by two-dimensional polyacrylamide gel electrophoresis, which were not detectable in the extracts from healthy trees.

Symptomless pear (Pyrus bretschneideri) in Liaoning province were inoculated to healthy apple tree (var. Ralls Janet) by grafting. About 80% plants showed leaf rolling, followed by leaves broken at the leaf stems, and rusty spots on tree stem. These symptoms resember to those caused by apple scar skin viroid. From these symptomatic stems an extra RNA was detected in the extracts using two-dimensional electrophoresis, which is not detectable in the extracts from healthy ones. The size of the extra RNA was estimated by bidirectional electrophoresis after denaturation. Its migration was the same as apple scar skin viroid RNA-1.

These results imply that pear may be a latent host of apple scar skin viroid. The relationships between pear rusty skin RNA with apple scar skin viroid is to be studied.

OP20.1

STUDIES OF TWO DIFFERENTIALLY HEAT-STABLE BACTERIOPHAGES BY CIRCULAR DICHROIC SPECTROSCOPY AND ELECTRON MICROSCOPY.
H. Marie Lawrence and Jeffrey A. Sands. Department of Biology, Lehigh University, Bethlehem, Pa. USA.

A comparative characterization of two recently isolated bacteriophages of the actinomycete, Thermomonospora fusca is presented. The phages, Tf2 and Tf3, are heat tolerant but differ in their absolute thermostabilities at high temperature. While both phages retain full infectivity at 60°C for more than 60 min., Tf2 shows no loss of infective capability after 60 min. at 80°C. Tf3 shows less than 0.1% survival after only 30 min. at 80°C. The phages are similar morphologically with icosahedral heads and non-contractile tails, although the length of the tails differs.

Circular dichroic spectroscopy (CD) studies were performed on the two intact viruses before and after various thermal treatments to monitor any structural transitions occuring in response to temperature change. The CD spectra are complemented by electron microscopic morphological studies performed on similar thermally-treated samples.

OP20.2

MORPHOGENETIC INTERMEDIATES OF ALKYLATED T3 and T7 BACTERIOPHAGES. B. Karska-Wysocki and M.D. Mamet-Bratley. Dép. de Biochimie, Fac. de Médecine, Univ. de Montréal, Montréal, Canada.

Lysates of Escherichia coli B cells infected with alkylated T7 or T3 phage contain phage tails and tail-related particles. When lysates were analyzed by sucrose gradient centrifugation, those fractions which contained tails and tail-related particles also contained bacterial proteins of molecular weight (as determined by SDS polyacrylamide gel electrophoresis) about 55,000. To better understand the role these bacterial proteins may play in phage morphogenesis, we have tested two hypotheses: 1) these proteins are involved in tail assembly; 2) they are associated with phage DNA metabolism. Our experimental results show that it is not the first but the second hypothesis which is probably correct. With mutants defective in tail formation, tail proteins disappeared from the gradient fractions but the bacterial proteins were still present. Furthermore phage DNA was found in the gradient fractions which contained both phage tails and the bacterial proteins. We therefore suggest that these proteins may play a role in the replication or encapsidation of phage DNA.

OP20.3

BACTERIOPHAGE T5: CLONING AND IDENTIFICATION OF A STRUCTURAL GENE. D. Kay and A.E. Wakefield. Sir William Dunn School of Pathology, University of Oxford, Oxford OX1 3RE. UK.

Bacteriophage T5 DNA was cleaved with Hind III and the fragments were inserted into plasmid pUC18. One transformant was found which contained the two non-adjacent fragments L and P (3868, 697 bp). Fragment L was isolated and inserted into pUC18 in both orientations giving two transformants, pAW4 and pAW5. These were transferred to E. coli F' which is a rapid-adsorption host for phage T5. Both F'(pAW4) and F'(pAW5) supported plaque formation of the T5 amber mutants am28 and am172 which are in the same complementation group and are known to be deficient in head synthesis. These mutants were isolated by Lanni (Kay & Wakefield, 1986, J.Gen. Microbiol. 67, 1663-1672).

E. coli F'(pAW4) was infected with T5 am172 and after incubation for 40min was subjected to PAGE followed by immunoblotting with anti-T5 serum. The bound antiserum was located with ^{125}I-goat anti-rabbit serum. One protein band was present in the E. coli F' bearing the plasmid and absent in the infected E. coli F' not carrying the plasmid. Its molecular weight was about 28,000 and corresponded to the minor head band, 8b, of Zweig & Cummings (1973, Virology 51, 443-453).

Having cloned the gene encoding p8b we are in a better position to investigate the function of this minor structural protein.

OP21.1

CAPRINE ARTHRITIS ENCEPHALITIS VIRUS: CHARACTERISATION OF PROTEINS. L. Belov and J.M. Whalley, School of Biological Sciences, Macquarie University, Sydney, Australia.

The proteins of an Australian isolate of the lentivirus caprine arthritis encephalitis virus (CAEV) were characterised by polyacrylamide gel electrophoresis and by Western blotting using immune goat sera. Monoclonal antibodies (MAbs) were produced to several CAEV structural proteins and were used to establish serological relatedness between virion polypeptides and their cellular precursors.

MAbs to p24, the main core protein, reacted also with p55, p41 and p33, while a MAb to p14 reacted also with p55, p41, p18 and faintly with p21 and p27, indicating that these proteins may be precursors or intermediates in the processing of the structural core proteins.

These MAbs were used to detect viral antigen in infected cells by fluorescent antibody labelling, providing a basis for further studies on CAEV infection in cell culture and for diagnosis of disease in affected animals.

OP21.2

DETECTION BY ELISA OF RABBIT ENTERIC CORONAVIRUS (RECV) VIRAL ANTIGEN PRODUCED IN HRT 18 cells and RABBIT MACROPHAGES. M.M. ENNAJI, M. TRUDEL, P. TALBOT, D. LACHANCE and J.P. Descôteaux. Centre de recherches en virologie, Institut ARMAND-FRAPPIER LAVAL, Quebec, CANADA. H7N4Z3.

RECV particles were observed in feces of Rabbit showing clinical signs of Enteritis. The virus was adapted in tissue culture using HRT 18 cells and Rabbit macrophage. The virus was also cloned under methyl-cellulose. The cells were infected with RECV and observed daily. The supernatant was Then colected, clarified by centrifugation and evaluated by enzyme Linked immuno sorbant assay (ELISA). The solide-phase Immuno assay employed microtiter plates coated with 0.025mL well of viral or control antigen over night at Room temperature. The FREE binding sites were bloked with the ELISA diluent. Antigen coated plates were used for antibody binding assay after one hour incubation at Room temperature. The primary antibody was the anti-RECV produced in guinea pig. The secondary antibody was peroxydase conjugated goat anti-guinea pig IgG. The substrate solution(0,1 mL) consisted of hydrogen peroxyde and 2,2 mM O-phenylene-diamine in 0,1 M sodium citrate buffer, PH 5,0. The enzyme reaction was terminated after 30 min. by adding 0,1mL of 1 N HCL into each well. ELISA showed viral antigens in the infected cell supernatant in both systems; and were confirmed by electron microscopy and by Immune electron icroscopy. The clones were also screens by ELISA.

OP21.3

A NEW LATEX AGGLUTINATION TEST FOR THE DETECTION OF ANTIBODY TO THE HUMAN IMMUNODEFICIENCY VIRUS (HIV). D.A. Fuccillo*, I.C. Shekarchi*, D.L. Madden, J.L. Sever, *Microbiological Associates Inc. and NINCDS NIH Bethesda, MD.

We have developed a new latex agglutination test (LA) for the detection of antibody to the causative agent of acquired immune deficiency syndrome (AIDS). HIV antigen is bound to latex through the use of the avidin-biotin technique. Avidin is covalently bound to latex beads and then biotinylated antigen is added at a predetermined dilution. Since each avidin can bind up to four biotin molecules, various concentrations of antigen can be attached without the possibility of inactivation which can be caused by bridging agents which are used for direct covalent attachment. The avidin-biotin latex agglutination (ABLA) test has a number of advantages over other serological assays. It is rapid, simple; does not require extensively trained personnel nor sophisticated equipment; has a long shelf life, and relatively low cost. This assay should be very useful for physicians offices, small laboratories, blood banks and for routine use in developing countries.

OP21.4

DIAGNOSIS OF A PERSISTENT VIRAL INFECTION IN PAGET'S DISEASE OF BONE WITH CONCOMITANT GIANT CELL TUMOR USING ANTIGEN DETECTION. B. Mills and F. Singer, Bone Physiology and Endocrinology Laboratories, Orthopaedic Hospital, Faculties of Dentistry and Medicine, U. of So. Calif., Los Angeles, CA, USA.

Characteristic nuclear and cytoplasmic inclusions associated with antigens of measles virus (MV) and respiratory syncytial virus (RSV) have been reported in the osteoclasts of more than 31 patients with Paget's disease of bone. Persistence of Paget's disease over many years is well documented by X-ray and surgical pathology. We now report the 34 year course of a patient with polyostotic Paget's disease of bone in whom the presence of a giant cell bone tumor was followed for 32 years. Ultrastructural evidence of persistent nucleocapsid-like structures was proven by biopsy 4 times over an 8 year period. The giant cells of the bone tumor contained the same nuclear inclusions and antigens identified by IFA as in the Paget's lesions. MV and RSV antigens were transferred to Wi 38 cells after cells from the giant cell tumor were cocultivated with Wi 38 cells. Injection of these Wi 38 antigen bearing cells into the tibiae of athymic mice produced osteosarcomas containing nuclear inclusions and antigens to RSV and MV. These nude mouse osteosarcoma cells cultivated in vitro also contained antigens to RSV and MV. It is concluded that a possible defective or mutant virus related to MV and RSV persisted in this patient for at least 8 years and may have been present for as long as 34 years.

OP21.5

DIAGNOSTIC RAPIDE DES INFECTIONS A VIRUS RESPIRATOIRE SYNCYTIAL.

Z. Kadi, S. Dali, S. Bakouri et A. Bouguermouh. Service de Virologie. Institut Pasteur et service de Pédiatrie. CHU Chéraga. Alger, Algérie.

A l'occasion d'une épidémie d'infection par le virus respiratoire syncytial (VRS) dans un service de Pédiatrie, le diagnostic a été fait par immunofluorescence à l'aide d'anticorps monoclonaux (IFm) sur des frottis de prélèvement rhinopharyngé. Le VRS a été isolé sur cellules Hep2 et les anticorps titrés par Immunofluorescence indirecte sur cellules Hep2 infectées par une souche de VRS. Comparée à l'isolement, l'IFm s'avère sensible (89 %) et spécifique (75 %). Nous montrons que des prélèvements trouvés positifs par l'IFm et négatifs en culture cellulaire sont en réalité de vrais positifs. Dans ces conditions, le test IFm apparaît plus sensible et plus spécifique que la culture cellulaire particulièrement quand la chaine du froid n'est pas respectée lors du transport des prélèvements. Dans cette étude, le test d'IF pour le titrage des IgM n'est pas très sensible (34 %) particulièrement sur des sérums prélevés dans les quatre premiers jours de la maladie.

OP21.6

RAPID DIAGNOSIS OF INFLUENZA A USING A TIME-RESOLVED FLUOROIMMUNOASSAY (TRFIA) IN COMBINATION WITH CONVENTIONAL CELL CULTURE METHODS— W.H. Greene, R.F. Betts, H. Siitari, M.A. Menegus, Faculty of the Departments of Microbiology/Immunology and Medicine, University of Rochester, Rochester, NY, USA and Wallac Biochemical Laboratory, Turku, Finland.

In most clinical laboratories Influenza virus is isolated in primary monkey kidney cells (PMK) and detected by hemadsorption (HAD) in 3-5 days . Specific identification is accomplished by using a number of serological methods. In this study, we evaluated early hemadsorption in combination with TRFIA for the rapid detection and identification of Influenza A. Nasal wash (NW) specimens were obtained from symptomatic (<48 hours) college students during an outbreak of Influenza A H1N1; 0.2 ml was inoculated into 3 tubes each of PMK. The remaining NW was frozen at -70°C and later titrated in MDCK cells. Inoculated tubes were incubated on a roller drum at 33°C. HAD was performed on days 1—3 post inoculation by removing the supernatant fluid (SF) and adding 0.5 ml of a 0.5% suspension of guinea pig RBC to the cell monolayer (CM). After 20 minutes at 4°C, HAD to the CM was scored as follows: (±) <10 positive cells, (+) >± but <10%, (1+) 10-25%, (2+) 26-50% and (3+) >50%. The CMs (with the RBC suspension) and the SFs were then frozen separately at -20°C; TRFIAs were later performed on each. TRFIA results were expressed as a ratio of the mean counts per second (CPS) of duplicates of the test specimen and mean CPS of 5 negative controls; ratios of ≥2 were considered positive.

Using HAD positivity by day 3 to define infection, specimens from 22 of 37 patients were positive for Influenza A; no virus was isolated from 15. Virus titers in NW specimens ranged from <1.0 to 4.5 (log 10) TCID50/ml; 5 were < 2.0. All cultures from influenza infected patients were HAD positive on day 1. The degree of HAD was low (± or +) for 10/22 and moderate to high (≥1+) for 12/22. TRFIA was positive in 21/22 (95.5%) of the HAD+ CMs while only 4/22 (18.2%) of the corresponding SFs were positive. All CMs and SFs from influenza negative patients were TRFIA negative.

In summary, despite the low virus concentration present in some specimens, early hemadsorption and TRFIA of lysed CM provided a sensitive and specific method for the rapid diagnosis of Influenza A infection.

OP21.7

PERFORMANCE OF AN ENZYME-IMMUNOASSAY(EIA) FOR DETECTION OF HERPES SIMPLEX VIRUS (HSV). C. Sherlock, J. Dragavon, L. Corey. Department Laboratory Medicine, University of Washington, Seattle, U.S.A.

The presence of HSV was assayed in 247 lesions from 122 male and 120 female patients attending an STD clinic. Lesion swabs were cultured in fetal fibroblast cells for up to 14 days and assayed for HSV proteins by a recently developed EIA (Abbott). Positive cultures were confirmed by monoclonal antibodies and optical densities read by Quantum II (Abbott). 79 of 91 culture +ve specimens were EIA +ve (sensitivity 87%) and 133 of 156 culture -ve specimens were EIA -ve (specificity 85%). Positive and -ve predictive values and accuracy were 77%, 92% and 86% respectively. Analysis of discordant results revealed no differences related to sex, first or recurrent episodes, type or site of lesion except that more vulval than penile lesions gave discordant results ($p<0.05$). When data from a second swab were available (culture, IFA), 8 of 16 (50%) apparently false +ve EIA results were judged to be true +ves; also false +ves occurred more frequently in lesions ≥ 3 days old vs. ≤ 2 days ($p<0.005$). Although the unrefined EIA predictive values are inadequate compared with culture, analysis of discordant results shows that the EIA is probably detecting HSV antigen in a significant number of culture -ve lesions and may prove to be a useful adjunct to culture for diagnosis of HSV lesions.

OP21.8

PRODUCTION OF MONOCLONAL ANTIBODIES TO TOMATO SPOTTED WILT VIRUS. J. L. Sherwood[1], M. R. Sanborn[2], G. C. Keyser[1], and L. D. Myers[1]. Department of Plant Pathology[1] and Botany and Microbiology[2], Oklahoma State University, Stillwater, Oklahoma 74078-0285, USA.

Stable hybridoma cell lines secreting monoclonal antibodies (MAB) to tomato spotted wilt virus (TmSWV) were produced by fusing spleen cells from BALB/c mice immunized with an isolate of TmSWV from Oklahoma to mouse myeloma cell line P3x63Ag8.653. Hybridoma clones produced antibodies of the IgG_1, IgG_{2a}, and IgG_{2b} subclasses. In a modified PAS-ELISA (Edwards; J. Virol. Methods 11:309), in which polyclonal rabbit antibody (TmSWV polyclonal serum provided by D. Gonsalves, Cornell University, Geneva, NY) was used as the capture antibody and the anti-TmSWV MAB as the probe, the MAB to TmSWV detected two isolates of TmSWV, but did not react to tobacco mosaic virus, brome mosaic virus, cucumber mosaic virus, wheat soilborne mosaic virus, tobacco streak virus or to five potyviruses tested. A dot-immunobinding assay with the MAB could detect TmSWV in a 1/128 dilution of infected Datura stramonium tissue. In a direct sandwich ELISA, with the MAB used as the coating antibody and alkaline phosphatase linked MAB used as the probe, TmSWV could be detected in over a 1/1000 dilution of infected D. stramonium tissue.

OP21.9

IMMUNOLOGICAL CHARACTERISTICS OF POTEX-, POTI- AND CARLAVIRUSES.

R.V. Gnutova, I.I. Sibiryakova, N.N. Kakareka, N.V. Rubleva, V.G. Korsh, Z.N. Kozlovskaya and V.P. Tolkach.

Institute of Biology and Pedology, Far-Eastern Science Centre of the Academy of Sciences of the USSR, Vladivostok, USSR.

The immunochemical studies carried ont using immunodiffusion tests, virobacterial agglutination rocket immunoelectrophoresis, "sandwich" and blocking methods of ELISA and immune electrone microscopy allowed to consider potato aucuba mosaic virus as a typical representative of the potexgroup as like studied by us potato virus X, hydrangea ringspot virus and white clover mosaic virus.

Identical antigen determinants were found in the following viruses of potygroup: bean yellow mosaic, potato viruses A and Y, onion yellow dwarf virus, turnip mosaic, soybean mosaic virus not identified previously.

The serological affinity of potato viruses M and S was not discovered using the method of immune electron microscopy.

OP21.10

NUCLEIC ACID HYBRIDIZATION FOR THE DETECTION OF CELL CULTURE AMPLIFIED ADENOVIRUS. C. Huang, R. Deibel, Wadsworth Center for Laboratories and Research, New York State Department of Health, Albany, N. Y., U.S.A.

Adenovirus DNA was spotted onto a nitrocellulose filter and hybridized with three ^{32}P-labeled DNA probes: total adenovirus type 2 DNA, subgroup C (total Ad2); and two recombinant plasmids, pAd2-H, containing the hexon gene, and pAd2-F, containing the fiber gene. Total Ad2 and pAd2-H detected with similar sensitivity 13 reference serotypes of the 5 genomic subgroups A through E. However, pAd2-H requires less preparation time than total Ad2. Plasmid pAd2-F hybridized only with serotypes from the homologous subgroup C. Of 61 patients' isolates amplified in KB cells, pAD2-H detected 100% of 32 isolates of the homologous subgroup C and 90% of 26 isolates from heterologous subgroups A, B and D. The detection rates with pAd2-F with the same isolates were 100% (subgroup C) and 0% (A, B, D). The demonstrated group specificity (pAd2-H) or excellent subgroup discrimination (pAd2-F) are of diagnostic utility. A biotinylated pAd2-H probe detected adenovirus but with less sensitivity: 60-100 pg of homologous DNA vs. 10-50 pg for the ^{32}P-labeled probes. Some specimens had sufficient virus to be detected by ^{32}P-labeled pAd2-H in direct tests on the clinical sample, but amplification is advisable for maximum sensitivity.

OP21.11

COMPARISON OF MOUSE MONOCLONAL ANTIBODIES AND RABBIT POLYCLONAL ANTISERUM FOR DETECTION OF RICE GRASSY STUNT VIRUS FROM INFECTED PLANTS. H. T. Hsu and H. Hibino. United States Department of Agriculture, Agricultural Research Service, Beltsville Agricultural Research Center, Beltsville, Md. 20705 USA and the International Rice Research Institute, Los Banos, The Philippines.

Serological detection of rice grassy stunt virus (RGSV) with mouse monoclonal antibodies and rabbit polyclonal antibodies was compared by enzyme-linked immunosorbent assay (ELISA), dot-blot immunoassay and latex agglutination test. A dilution end point of about 10^{-3} was obtained for both mouse monoclonal antibodies and rabbit polyclonal antiserum by latex agglutination tests. Similar sensitivities were attained in dot-blot immunoassay when alkaline phosphatase was used. Use of peroxidase-labelled antibodies was unsuccessful due to a positive reaction of healthy plant sap in dot-blot immunoassays. A ten-fold increase of sensitivity of RGSV detection was achieved by using double antibody sandwich ELISA. Non-specific reactions due to the presence of anti-host component immunoglobulins commonly observed in tests with rabbit polyclonal antiserum were eliminated when mouse monoclonal antibodies were used in ELISA and dot-blot immunoassays. For practical purposes, however, the latex agglutination test provides the simplest procedure and most economical method for routine detection of RGSV.

OP21.12

DOT ENZYME IMMUNOASSAY FOR THE DETECTION OF ANTIBODIES TO COMMON MURINE VIRUSES. J. van der Logt, R. van Boesschoten, F. Heessen, and A. van Loon. Dept. of Med. Microbiology, University of Nijmegen, Nijmegen, The Netherlands.

A dot enzyme immunoassay (DIA) was evaluated and compared with a standard ELISA in microtitre plates for the serological diagnosis of infections with the common mouse viruses sendai, pneumonia virus of mice and mouse hepatitis virus. In the DIA the antigen and control antigen were spotted in one line on nitrocellulose (NC) sheets with an imprinted 3-mm grid. Sera were applied by saturating absorbent paper strips and placing them on the NC sheets over the antigen dots. Sheets were then incubated with anti-mouse IgG conjugated with peroxidase and substrate. Interpretation of the results was easily made by visual inspection of reaction and by using a weakly positive control serum as a reference to determin the positive/negative cut-off value. There was no significant difference in sensitivity and specificity between the DIA and the ELISA in detection of natural or laboratory infection.

Because of its ease of performance and high sensitivity and specificity the DIA could be an excellent test to be executed in laboratory with limited equipment for monitoring laboratory animals in one test for antibodies against a panel of viruses.

OP21.13

Reactivity to the HIV proteins in sera of asymptomatic carriers, LAS-ARC and AIDS patients. Giorgio Achilli*, E. Cattaneo*, M. Danesino*, M. Marchisio*, L. Minoli*, E.G. Rondanelli*. Department of Infectious Diseases, IRCCS - San Matteo, Pavia Italy* - Labor Research Laboratoires, Milan Italy**.

We analyzed by the Western-blot technique the patterns of reactivity to the HIV protein p17, p24 and gp41 in sera from asymptomatic HIV IgG positive carriers LAS-ARC and AIDS patients. All 34 AIDS patients were positive for antibodies to gp41, 96.8% to p24, and 90% to p17. In the LAS-ARC group (32 cases) 90.6% patients were positive to gp41, 81.3% to p24, and 90.7% to p17. The distribution among asymptomatic carriers (66 cases) was: 100% to gp41, 96.4% to p24, and 81.8% to p17. We observed in the three group under study singificant differences in reactivity to the marker proteins. It appears that the three stages of the diseases do not necessarily represent an evolutionary process of the HIV infection. Among the LAS-ARC patients 10.7% of sera are not reactive to gp41; while 100% of asymptomatic carriers and AIDS patients have antibodies to this protein. Moreover we observed a decrease in positivity to p 24 from asymptomatic carriers to LAS-ARC, and to AIDS patients. Therefore LAS-ARC patients negative to gp41 antibodies could be considered at low risk to develop AIDS.

Partially supported by funds "Ricerca corrente 1986" IRCCS - San Matteo Pavia, Italy.

OP21.14

PRODUCTION AND CHARACTERIZATION OF MONOCLONAL ANTIBODIES AGAINST THE THREE SEROTYPES OF POLIOVIRUS. P.Payment, J.Lecomte and M.Trudel. Virology Research Center, Institut Armand-Frappier, Laval, Quebec, Canada, H7N 4Z3.

The differentiation of poliovirus serotypes as well as the evaluation of the antigenic relationships between the three serotypes of poliovirus is possible through the use of adsorbed antisera or better, monoclonal antibodies (MAb) with specific reactivities. We have prepared mouse MAb by fusion of immunized mouse spleen cells with mouse myeloma cells (AgX63-653). Mice were immunized with vaccine (Sabin 1, 2 or 3) or wild (Mahoney, MEF-1 or Saukett) reference strains. Hybridomas were screened by neutralization against homologous and heterologous serotypes as well as against a panel of 30 well-characterized poliovirus strains. MAb reactive with all the homologous serotype strains were obtained. For serotype 1 and 3 its was possible to obtain MAb differentiating vaccine and non-vaccine strains. For serotype 2, it was not possible to obtain such a clear-cut differentiation: its was possible however to obtain two MAb with patterns of reactivity permitting the differentiation of vaccinal from non-vaccinal strains. These MAb will permit the rapid and certain characterization of the antigenicity of new isolates from clinical cases as well as from environmental sources.

OP21.15

DETECTION OF HEPATITIS B SURFACE ANTIGEN USING EU-LABELED MONOCLONAL ANTIBODIES. H. Siitari, P. Laaksonen. Wallac Biochemical Laboratory, Turku, Finland.

A highly sensitive one-incubation immunoassay for hepatitis B surface antigen has been developed. The high sensitivity has been achieved by using Eu-labeled monoclonal antibodies and time-resolved fluorescence measurement. 12-well microtitration strips were coated with polyclonal anti-HBsAg antibodies. Serum specimens were added together with the assay buffer containing the Eu-coupled monoclonal antibodies, and incubated for 30 min at 40°C. After washing the bound Eu was measured in enhancement solution (Delfia^{R}, LKB-Wallac, Turku, Finland) in an Arcus^{R} fluorometer (LKB-Wallac, Turku, Finland).

Time-resolved fluorescence measurement of the Eu 2-trifluoroacetone chelate guarantees that any nonspecific background signal originating from the sample or scattered light is avoided. This is possible because the time-resolved fluorometer discriminates the short-lived (<100 ns) background fluorescence from the specific long-lived (~1000 µs) Eu-fluorescence and only the photons emitted from Eu are collected and expressed as counts per second. The measurement time is one second per sample. With this method HBsAg can be measured with very high sensitivity. Moreover, the simple assay procedure together with the rapid measurement provides the results in a short period of time even from large scale runs.

OP21.16

ASSAY OF RHABDOVIRUS INFECTIONS IN PLANTS USING PLANT LECTINS. G. Adam and T.C. Bog-Hansen. Inst. for Biology, University of Stuttgart, F.R.G. and The Protein Laboratory, University of Copenhagen, Denmark.

The glycoproteins of plant rhabdoviruses could serve as marker proteins, which when present in plant extracts, indicate the infection with a rhabdovirus. Since lectins specifically bind to glycoproteins, we have tested the possibility to detect rhabdovirus glycoproteins with biotinylated lectins after separation of crude extracts from plants on polyacrylamide gels and blotting of the separated proteins on nitrocellulose.

Among the 13 lectins tested, 6 were found to react with the glycoproteins from potato yellow dwarf virus or eggplant mottled dwarf virus. Two of the 6 positive-reacting lectins, STA and WGA, were extremely suitable as specific probes for the viral glycoproteins, since they did not react with glycoproteins from host origin, having the same electrophoretic mobility on the gels. The lectins could detect the viral glycoproteins up to a dilution of 1:128 of the plant extracts, whereas a virus-specific antiserum was approximately eigth-times more sensitive. A differentiation between the two rhabdoviruses in our studies by the lectins was not possible.

The applicability of the proposed assay for rhabdovirus infections with lectins was tested further with strawberry crinkle virus, a plant rhabdovirus for which hitherto no quick assay exists.

OP21.17

FLUOROGENIC ENZYME IMMUNOASSAY ON NITROCELLULOSE MEMBRANES FOR QUANTITATION OF NEWCASTLE DISEASE VIRUS ANTIGENS. R.E. Fulton, J.P. Wong, Y.M. Siddiqui and H.-S. Tso. Defence Research Establishment Suffield, Ralston, Alberta, Canada.

A highly sensitive fluorogenic enzyme-linked immunosorbent assay (FELISA), which utilized nitrocellulose membranes as solid phase support, was developed for the detection and identification of Newcastle Disease Virus (NDV) antigens. Reagents were standardized and the theoretical lower limits of test sensitivity were compared, in "sandwich" and "indirect" formats, to those of a comparable chromogenic enzyme immunoassay. The "sandwich" FELISA exhibited maximum sensitivity and detected 10 fg of purified virus protein per ml (500 ag per test volume). Specificity of the FELISA was evaluated by challenging the system with heterologous strains of NDV and with other serologically related and unrelated viruses. In a clinical trial, virus was detected by FELISA directly in fecal samples of chickens undergoing vaccination with NDV. The test was simple and rapid, and detected virus antigens in crude samples at extremely low concentration.

OP21.18

DIRECT SEROTYPING OF HUMAN ROTAVIRUS IN STOOL BY ELISA USING MONOCLONAL ANTIBODIES: ITS COMPARISON WITH FLUORESCENT FOCUS NEUTRALIZATION TEST USING RABBIT ANTISERA. T.Urasawa,K.Taniguchi.Y.Horita,N.Kobayashi and S.Urasawa. Department of Hygiene, Sapporo Medical College, Sapporo, Japan.

Using the 4 monoclonal antibodies reacting specifically with VP7 neutralizing protein of 4 different serotypes of human rotavirus(HRV), we developed a method for serotyping HRVs by an enzyme-linked immunosorbent assay (ELISA-serotyping; J. Inf.Dis., in press). In the present study, serotype(sero.) of cell culture-adapted and non-adapted(stool) viruses was determined in fluorescent focus neutralization test with polyclonal rabbit sera (FA-NT-serotyping) as well as in ELISA-serotyping to assess the sensitivity and specificity of the 2 tests. As for 29 of 33 culture-adapted strains, both tests gave consistent results (11:sero.1,10:sero.2,5:sero.3 &3:sero 4). Discrepancy between the tests was ascribable to the fact that ELISA-serotyping could detect only the sero. antigen on VP7(but not on VP3) and that sero.3-specific monoclonal antibody failed to detect one sero.3 strain. Next,direct serotyping of HRV in stool was carried out by both tests using 72 stool samples confirmed to be positive for HRV by electron microscopy or the commercial kits for group A RV detection. ELISA-serotyping enabled us to serotype 58(81%) of 73 (31:sero.1,12:sero.2,4:sero.3 & 11:sero.4), while only 28 isolates (36.8%) could be serotyped in FA-NT-serotyping (14:sero.1,5:sero.2,2:sero.3 & 7:sero.4). The validity of ELISA-serotyping in clinical and epidemiological studies was confirmed.

OP21.19

RAPID DETECTION AND SUBGROUPING OF HUMAN ROTAVIRUSES BY ONE-STEP MONOCLONAL ANTIBODY-BASED ELISA ASSAYS. A. Saresini, M. Torsellini, A. Di Matteo, G. Gerna.
Virus Lab, Inst. of Infectious Diseases, University of Pavia, 27100 Pavia, Italy.

Using peroxidase-labeled monoclonal antibody (HRP-MAb) reactive to VP6 of group A rotaviruses, two one-step ELISA systems were developed for rapid (1h 15 min) detection and subgrouping of human rotavirus (HRV) strains in stool extracts. Polyclonal rotavirus antibody-coated microtiter wells were incubated for 1h with mixtures of stool samples and group or subgroup specific HRP MAbs. Two sequential incubations (1h each) were done in parallel. HRV detection by one-step ELISA had a sensitivity comparable to EM and conventional ELISA (taking 6h to complete), whereas HRV subgrouping was better done by two sequential incubations, since samples with low ODs gave equivocal results with the one-step method. Partially supported by Min.Sanità, IRCCS Pol. S.Matteo, Ric. corrente 1985 and by Reg. Lombardia, Ric.Finalizzata, Project no. 875.

OP21.20

IMMUNOFLUORIMETRIC ASSAY FOR DETECTION OF ANTI-HIV ANTIBODIES IN HUMAN SERA. F. Titti*, A. Acati*, P. Verani*, S. Buttò*, A. Sebastiani*, G.B. Rossi*. *Laboratory of Virology, Istituto Superiore di Sanità, Rome; **"La Sapienza" University, Rome, Italy.

A new immunoassay, time-resolved fluoroimmunoassay (Tr-Fia), has been standardized for detection of anti-HIV antibodies in human sera. This method is based on the use of a crude virus preparation coated on a polystirene microtiter plates and of a swine anti-human IgG labeled with a rare earth metal, europium, as fluorescent label.

A total of 475 selected sera were analyzed by Elisa commercial kit and by Tr-Fia. The prevalence of positivity was 29.6% by Elisa versus 26.7% by Tr-Fia. The sensitivity of both system was 100%. The specificity was 87.5% for Elisa, whereas it reached a value of 99.4% for Tr-Fia.

Tr-Fia is sensitive, specific and alternative test for anti-HIV antibodies detection which a) avoids consuming, expensive and hazardous sucrose density-gradient purification step, b) excludes the use of radiotracers or substrates with potential risk for operator for revealing the reaction, c) minimizes the number of false reactivities, and therefore the number of confirmatory tests, d) is easy to standardize. This method could be also applied for antigen detection in culture fluids as well as in human sera.

OP22.1

IN VIVO AND IN VITRO EFFECTS OF SPECIFIC ANTIBODIES ON MHV3 REPLICATION. Vera C.B. Cainelli Gebara and Carlos A. Pereira. Laboratório de Imunologia Viral, Instituto Butantan, São Paulo, Brasil.

In vivo and in vitro studies of MHV3 replication were carried out to elucidate a possible enhancement effect promoted by sub-neutralizing doses of specific antibodies. This mechanism may be involved in the pathogenesis of MHV3 infection where macrophages has been show to represent a crucial stage during the disease. Peritoneal macrophages were isolated from resistant A/J and susceptible BALB/c mice strains and assayed for virus replication in the presence of specific antibodies.A light enhancement effect was observed in these experiments. Notwithstanding, the inoculation of a mixture of MHV3 and specific antibodies renders resistant A/J mice susceptible to the virus infection. These animals die from acute hepatitis a few days after infection. Studies are now in progress to clarify if specific antibodies can promote any alteration of the virion specific polypeptides synthesis.

Financial support:Instituto Butantan,FAPESP,CNPq.

OP22.2

SUSCEPTIBILITY TO MHV3 IN GENETICALLY SELECTED HIGH AND LOW ANTIBODY RESPONDERS MICE (HR AND LR). M.A. Lucchiari, C.A. Pereira. Laboratorio de Imunologia Viral, Instituto Butantan, São Paulo, Brasil.

MHV3 constitute a model of viral infection in which resistance depends on the genetic background of the animal. It has been shown a direct relationship between the in vivo resistance and the ability of macrophages to partially restrict the in vitro MHV3 growth. The HR and LR mice show a difference in the macrophage activity that leads to difference in the magnitude of antibody response. During the infection, MHV3 growth in tissues was significantly enhanced in LR mice, which correlates with the higher titers observed in macrophages isolated from LR mice. This may explain the slightly lower titer of antibodies in LR mice and their higher susceptibility to MHV3 infection at limiting dosis of virus.

Financial support: Instituto Butantan, FAPESP, CNPq

OP22.3

PERSISTENT INFECTION OF CNS CELLS BY THEILERS MURINE ENCEPHALOMYELITIS VIRUS (TMEV). D. Stone, M.G. Rumsby. Department of Biology, University of York, York YO1 5DD, England.

TMEV causes a persistent CNS infection in mice which is accompanied by primary demyelination. The mechanism by which the virus persists in the CNS is unknown but recent studies have suggested that virus protein is restricted in nerve cells.

We have studied the replication of two avirulent strains (DA and BeAn) of TMEV which persist in the CNS and a virulent strain (GDVII) which is associated with a lethal acute disease. During infection of permissive BHK-21 cells ^{3}H-uridine incorporation into viral RNA in the presence of actinomysin D is reduced with DA and BeAn strains, and the incubation period prior to RNA synthesis is increased, compared with GDVII. In addition, separation by SDS-PAGE of ^{35}S-methionine labelled polypeptides from DA and BeAn infected BHK cells reveals that expression of virus capsid protein is delayed compared with GDVII infection where synthesis of the capsid protein is rapid.

The replication of these TMEV strains in mixed brain cell cultures is now being examined to elucidate the mechanism of viral persistence in the CNS.

OP22.4

VIRAL ENCHANCEMENT OF EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS (EAE): A CONSEQUENCE OF IMMUNE CELL INFECTION? B. Clark, A.J. Suckling. Department of Biology, University of York, York YO1 5DD, England.

In studying the mechanism by which neurotropic viruses potentiate the induction of EAE we have noted that infection of guinea pig mononuclear cells with an avirulent form (A7) of Semliki Forest Virus (SFV) depresses their ability to undergo blast transformation in response to both mitogen and antigen stimulation. In experiments to determine the mechanism of this inhibition results have been obtained which suggest that under certain circumstances SFV produces a productive infection of a restricted population of cells of the lymphocyte lineage which are then phagocytosed by macrophages. In terms of the enhancing effect of virus infection on the induction of EAE two possibilities arise. The first is that the restricted population of cells which are infected perform a modulatory function in the generation of an immune response and that their loss allows subsequently induced responses to proceed unregulated. The second is that macrophages activated during the course of the infection express this reactivity against unrelated antigens and therefore show an increased ability to initiate an immune response following inoculation of animals for EAE. Both possibilities will be explored.

OP22.5

DETECTION OF NUCLEAR ANTIGEN IN FLAVIVIRUS INFECTED CELLS. A. Buckley & E.A. Gould. Arbovirus Research Unit, London School of Hygiene and Tropical Medicine, United Kingdom.

Two monoclonal antibodies specific for either the viral envelope glycoprotein or the non-structural(NS1) glycoprotein were derived from West Nile and yellow fever viruses respectively. Their antigenic reactivity with a large selection of flaviviruses was tested by indirect immunofluorescence microscopy. Both produced cytoplasmic fluorescent staining patterns with the homologous virus from which they were derived.

Two unexpected patterns of fluorescent labelling were observed with Zika and Langat virus. One antibody produced fluorescent staining of the nuclei but not the cytoplasm of cells infected with Zika virus whereas the other antibody labelled only the nucleoli of cells infected with Langat virus.

Further experiments showed that this effect was virus specific. The significance of these results will be discussed.

OP22.6

A STRUCTURAL ALTERATION IN THE ENVELOPE (E) PROTEIN OF YELLOW FEVER (YF) VIRUS ANALYSED IN VIVO. N. Cammack, and E. A. Gould, Arbovirus Research Unit, Winches Farm Field Station, 395 Hatfield Road, St Albans, England.

Physical and functional maps of the E protein of YF17D vaccine virus and the wild-type parent Asibi strain were prepared using eleven YF17D E-specific monoclonal antibodies (MCA). Reciprocal competition binding enzyme-linked immunosorbent assays (CB/ELISA) defined a major antigenic domain of five overlapping flavivirus group-reactive MCA sites that was conserved on both viruses. Evidence based on haemagglutination-inhibition (HI) data, implicated structural rearrangements between the two virus E proteins. Furthermore, proximal to the major domain on the 17D vaccine strain, MCA 427 defined a neutralisation (N) site overlapping the epitopes for MCA 868 and MCA 813 only. On the wild-type parent Asibi strain, the disposition of this site was altered; MCA 427 did not react in N tests using YF Asibi. In vivo examination of this structural alteration demonstrated that MCA 427 was unable to prevent lethal infection of mice by either virus but in fact, enhanced the infectivity of the wild-type YF Asibi virus.

OP22.7

CONCURRENT INFECTION OF HUMANS BY YELLOW FEVER AND COWBONE RIDGE VIRUSES. E.A. Gould, A. Buckley, P.A. Cane & J.S. Porterfield*. Arbovirus Research Unit, London School of Hygiene and Tropical Medicine, United Kingdom. *Sir William Dunn School of Pathology, University of Oxford, Oxford, United Kingdom.

During an investigation of an outbreak of jaundice in Nigeria in 1952 suspected as being due to yellow fever, a virus was isolated from a 10 year old African girl, Chuku, who presented with fever and headache but was anicteric. The virus was initially identified on the basis of mouse protection tests as Zika virus. This was questioned by Clarke who claimed that it was more closely related to Spondweni virus. We present evidence based on plaque purification and tests with monoclonal antibodies that "Chuku virus" is actually a mixture of two flaviviruses, yellow fever and a virus closely related to Cowbone Ridge virus.

These observations are of interest for several reasons. They suggest that the child was concurrently infected with two viruses the second of which was not neutralised by yellow fever antiserum so that yellow fever was not diagnosed, although epidemiological evidence indicated that yellow fever virus was active in the area at that time. This isolation antedates those of Spondweni virus in South Africa in 1955 and Cowbone Ridge virus in Florida in 1965.

OP22.8

PASSIVE PROTECTION OF MICE WITH NON-NEUTRALIZING ANTIBODIES IN ANTIVIRAL ANTISERUM AGAINST VIRAL ENCEPHALITIS.

K. Yamamoto[1] and B. Simizu[2]. [1]Dept.of Virol.& Rickettsiol., National Institute of Health, Tokyo, Japan. [2]Dept.of Microbiol., School of Medicine, Chiba Univ., Chiba, Japan.

It has been suggested that the protection of animals passively immunized with some monoclonal antibodies (McAbs) from viral infection is not always parallel to the neutralizing (NT) activity of antibodies (Abs). However, it cannot guarantee that Abs having properties like the non-NT McAbs are dominant in immunized animals. In this study, we attempted to determine whether a conventional antiserum (AS) from animals immunized with whole virions contains the non-NT Abs which protect animals from viral infection by passive immunization.

When a diluted AS was injected into mice and challenged by western equine encephalitis virus, it was found that the mice carrying the sub-NT level of the Ab were protected from viral encephalitis. When mice were challenged with a virus preincubated with highly diluted Abs containing NT titer less than 1 before injection, it was found that the mice receiving the virus-Ab complex also showed no clinical sign of infection. These results suggest that the conventional AS contains non-NT but protective Abs which are distinct from NT Abs.

OP22.9

CHEMOTAXIS OF POLYMORPHONUCLEAR LEUKOCYTES TO VARICELLA-ZOSTER VIRUS ANTIGEN

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Polymorphonuclear leukocytes (PMNs) predominated in the vesicular fluid and adherent to the vesicular base. In the present studies, mechanisms of migration of PMNs in the vesicular fluid were examined.

PMNs were isolated from healthy adults with centrifugation using a mono-poly resolving medium. The varicella-zoster virus (VZV) antigen was prepared with sonication of VZV-infected human embryonic fibroblasts (HEF). The control antigen was prepared with sonication of uninfected HEF. Chemotaxis of PMNs was performed with the filter-chamber method.

Chemotaxic index of PMNs in the presence of the VZV antigen was 26.8 ± 2.9 (n=8), while that in the presence of the control antigen was 8.3 ± 3.7 (n=5). Chemotaxis of PMNs was inhibited when the VZV antigen was pretreated with a seropositive sera. Meanwhile, seronegative sera was unable to inhibit chemotaxis of PMNs. Chemotaxis of PMNs was also inhibited when the VZV-antigen was pretreated with monoclonal antibodies to VZV GP-I and GP-II. However, a monoclonal antibody to GP-IV was not able to inhibit migration of PMNs. These results suggested that the VZV antigen played a role of a chemoattractant, and GP-I and GP-II might be its major components

OP22.10

NATURAL KILLER ACTIVITY AGAINST VARICELLA-ZOSTER-VIRUS INFECTED TARGETS IN PATIENTS WITH LEUKEMIA

Masahiro Ito,Yutaka Ota,Machiko Imaji,Sadayoshi Torigoe Hitoshi Kamiya and Minoru Sakurai

Department of Pediatrics, Mie university, Tsu, Japan

Natural Killer (NK) cells spontaneously lyse tumor cells and virus infected fibroblasts. VZV infection in children with leukemia and other malignancies becomes severe, but the mechanism is still obscure. NK cells , especially against VZV-infected targets, may have an important roles to recover from VZV infection.

In this study we tested NK activity against VZV infected targets in 40 patients with leukemia. In patients who were receiving chemotherapy within 1 year after onset, NK activity (mean=17.5) were significantly decreased comparing with patients who had completed chemotherapy (mean=32.2). Then we investigated the percentage of Leu 11b positive cells, percent conjugates (effector cells binding to target cells), interferon titer in the supernatants in NK assays. In some patients with low NK activity, percentage of Leu 11 b positive cells,percent conjugates, and interferon production were low. However there were no significant correlation between NK activity and other factors.

These results suggest that: (1) NK activity against VZV infected targets were significantly decreased in patients who were receiving chemotherapy. (2) NK cells have an important roles in VZV infection (3) Mechanism of decreased NK activity might show the lytic capacity of NK cells.

OP23.1

STRUCTURE AND FUNCTION OF CAULIMOVIRUS GENE PRODUCTS. Roger Hull, Department of Virus Research, John Innes Institute, Colney Lane, Norwich NR4 7UH, U.K.

Sequence data from several isolates of cauliflower mosaic virus (CaMV) and from carnation etched ring virus (CERV) were analysed. The gene I product of these caulimoviruses has amino acid sequence homology with tobacco mosaic virus P30 and thus it is suggested that this gene product is involved in cell-to-cell spread. The amino acid sequences of CaMV and CERV gene II product show two peaks of homology which correspond to two structural domains. This is compatible with the hypothesis that this gene product, the aphid transmission factor, links between whatever is transmitted (particles or inclusion bodies) and certain regions of the aphid's exoskeleton. Caulimovirus gene IV and V products show structural and sequence similarities to retrovirus gag and pol proteins respectively. Analysis of sequence homologies between CaMV and CERV gene V products clearly resolves three structural domains. The similarities between caulimovirus and retrovirus proteins are of significance in understanding the structure and function of caulimovirus replication complexes.

OP23.2

TRANSCRIPTION MAP OF TOMATO GOLDEN MOSAIC VIRUS. G. Sunter, W.E. Gardiner, S.G. Rogers*, D.M. Bisaro. Department of Botany and Microbiology, Auburn University, Auburn, AL 36849, and *Biological Sciences, Monsanto Company, St. Louis, MO 63198, USA.

Tomato golden mosaic virus (TGMV) is a whitefly-borne agent which belongs to the geminivirus group. The viral genome is divided between two ssDNA components, designated A and B, both of which are required for infectivity. Sequence analysis of TGMV DNA has identified a total of six open reading frames (ORFs); four on the A component and two on the B component. These are located on both strands of the dsDNA intermediates of each component, suggesting a bidirectional transcription strategy.

RNA isolated from TGMV-infected Nicotiana benthamiana plants contained a number of TGMV specific polyadenylated mRNAs. The transcripts identified were shown to be of the size and position predicted for the ORFs. Transcripts originated from both strands of each component, confirming bidirectional transcription. The 5' and 3' termini were mapped for each mRNA by primer extension and S1 nuclease analysis.

OP23.3

TRANSCRIPTION OF THE CASSAVA LATENT VIRUS GENOME. A. Ward, J. Stanley, R. Townsend. Department of Virology, John Innes Institute, Norwich, England.

The cassava latent virus (CLV) genome comprises two ssDNA circles. Sequence analysis shows six open reading frames (ORFs) with the potential to encode proteins of mol.wt. $\geq$10K which are strongly homologous with those of closely related geminiviruses. These are found in both the virion (+ve) and complementary (-ve) sense of both DNAs. Northern blots of RNA samples from Nicotiana benthamiana infected with CLV reveal five major, virus-specific species. Using nuclease-S_1 protection techniques these have been mapped to the +ve and -ve senses of both DNAs, confirming that transcription is bidirectional. With finer resolution mapping they have been demondtrated to encompass the six ORFs thought most likely to be expressed.

An intergenic region separates the positions of the 5' termini of +ve and -ve sense transcripts on both DNAs. Chimaeric viral constructs are being used to study the potential role of these sequences in transcription control and replication. These experiments have a bearing on the potential of geminiviruses as gene vectors

OP23.4

ELECTRON MICROSCOPY DETECTION OF A NEW REGULAR STRUCTURE WITHIN THE CAULIFLOWER MOSAIC VIRUS-INDUCED VIROPLASMS. D. Rodríguez, D. López-Abella, J.R. Díaz-Ruíz. U.E.I. de Fitopatología, Centro de Investigaciones Biológicas, C.S.I.C., Velázquez 144, 28006 Madrid. Spain.

Cells infected with Cauliflower Mosaic Virus (CaMV) contain cytoplasmic proteinaceous structures called viroplasms which are thought to be the sites of viral reverse transcription and virion assembly. The relationship between viroplasm development and virus replication is not well understood, and little is known about the nature of the environment formed within viroplasms which might favore reverse transcription and processing of viral proteins for the assembly and maturation of the particles.

A detailed ultrastructural study has been made on viroplasms induced by two CaMV strains, by both thin sectioning and negative staining electron microscopy techniques. Examination of negatively stained purified viroplasms resulted in the consistent detection of a previously undescribed virus-associated regular structure within the viroplasms matrix. The same structure has been also visualized in section of purified viroplasms and much less frequently in sections of infected cells.

The regular structure detected inside the viroplasms could represent an intermediate step involved in the process of the CaMV assembly.

OP24.1

EFFECTS OF RETROVIRUS INFECTION ON ENDOCRINE CELLS IN VITRO.
Y.A. Naiah, W. Turner, Department of Microbiology, Howard University, College of Medicine, Washington, D.C. 20059.
In an attempt to elucidate the role of viruses in certain endocrine disorders, an investigation of virus-endocrine cell interaction-in vitro was undertaken. Cell lines derived from murine adrenal glands (Y-1) and murine pituitary glands (GH-3) were infected with Moloney Murine Leukemia Virus (M-MuLV). Replication of the virus, as well as the effect of the virus on cell growth and function, was monitored. The yield of M-MuLV from infected Y-1 cells (2.6×10^4 FFU/ml) and GH-3 cells (2×10^3 FFU/ml) was significantly lower than yield of virus from infected NIH-3T3 cells (4.3×10^5 FFU/ml). M-MuLV infected Y-1 cells produced more virus per cell (1.627 FFU/cell) than infected GH-3 cells (0.714 FFU/cell). M-MuLV infected GH-3 cells (3.71×10^6 cells/culture and Y-1 cells (1.64×10^6 cells/culture) showed significant increase in total cell population compared to uninfected GJ-3 (1.33×10^6 cells/culture) controls. The rate of multiplication of infected GH-3 cells was 1.10/times greater than uninfected GH-3 controls, while the rate of infected Y-1 is approximately 2 times higher than uninfected Y-1 cells. Converely, infected Y-1 cells appear to multiply at rate (2.5 times) slower than infected GH-3 cells. Employing RIA, infected GH-3 cells showed approximately 35% reduction in prolactin secretion compared with uninfected cells. In contrast, steroidogenic hormone synthesis in Y-1 cells was 1.5 times greater in M-MuLV infected cells than uninfected controls.

OP24.2

ALTERATIONS IN THE GENOME OF A RETROVIRUS THAT HAVE EFFECTS IN CIS ON THE ENCAPSIDATION OF GENOMIC RNA.
J.R. Christensen, P. Balduzzi, P. Ooi. Department of Microbiology and Immunology, School of Medicine and Dentistry, University of Rochester, N.Y., U.S.A.

Experiments were designed to investigate the effect of alterations in the genomic RNA on the efficiency of encapsidation in the avian sarcoma virus, UR2, which has the smallest genome, 3165 nt., of any naturally-occurring retroviruses. UR2 contains no complete genes for replicative functions and expresses only the transforming protein P68gag-ros. We have made deletions in pUR2, a pBR322-cloned version of the provirus of UR2, in the region of the defective env gene. Each deletion has its 5' end at the PvuII site, 8 nt. downstream from the end of the ros sequence; the longest extends into the "DR" sequence, an analog of dr 1, which is present on each side of the src gene of RSV, and which has been shown by others to be necessary for encapsidation. Encapsidation efficiency was judged by the ability of chick embryo fibroblasts, transfected with plasmid DNA plus DNA of the cloned helper virus, to produce infectious transforming virus.

A deletion of 658 nt., which leaves DR intact, produces a very marked reduction in virus production, while one of 706 nt., which cuts into DR, produces a total defect. We interpret the effect of the 658 nt. deletion as being due to a size limitation on the efficiency of the encapsidation process, and will report on the effects of restoring normal genome size by the addition of "stuffer" DNA in place of the deleted DNA in the plasmid.

OP24.3

INSERTION OF MuLV-K VARIANT INTO THE CODING REGION OF β-LACTAMASE DOES NOT DISRUPT ITS EXPRESSION.
V. Dias-Ferrao and J. E. Knesek. Department of Biology, Texas Woman's University, Denton, Texas, U.S.A.

A foreign DNA with Pst 1 ends when cloned into the Pst 1 site of pBR322 plasmid, whose location is within the β-lactamase coding region, disrupts the expression of the β-lactamase gene.

Kirsten murine leukemia virus (MuLV-K) variant DNA was cloned into the Pst 1 site of pBR322. This recombinant molecule was used to transform Escherichia coli DH-5 cells. On screening, transformed bacteria were ampicillin resistant indicating that a functional β-lactamase gene product was produced in the bacterial cells which contained the recombinant variant MuLV-K:pBR322 molecule.

Furthermore, a deletion mutant of the variant MuLV-K: pBR322 allowed the bacteria to grow in the presence of ampicillin. No free pBR322 was isolated from the bacteria containing this mutant. Therefore, the mutant recombinant molecule appears to code for a functional β-lactamase gene product in spite of an interruption within the coding region of the gene. This hypothesis is supported by RNA analysis and nucleic acid sequencing data.

OP24.4

INTERACTION OF BONE-ACTIVE AGENTS AND MAV-2(0)
R. E. Smith, J. Torgensen, P. H. Long, J. K. Maurer. Department of Microbiology, Colorado State University, Ft. Collins, CO 80523 USA, and the Procter and Gamble Co., Cincinniti, OH 45247 USA.

Chick embryos and newly hatched chicks were given sodium fluoride (NaF), 1,25-dihydroxycholecalciferol ($1,25(OH)_2D_3$), bovine parathyroid extract (PTE), prostaglandin E_2(PGE_2), and epidermal growth factor (EGF) to establish maximal dose levels and toxic effects. NaF was tolerated at 300 ug administered three times to the embryo and at 1200 ppm in the feed of the hatched chick. $1,25(OH)_2D_3$ was tolerated at 10 ng/chick given as a single dose to the 14 day-old embryo or hatched chicks. When NaF and $1,25(OH_2)D_3$ were given to chick embryos and embryonic bones were examined at 18 days of incubation, cortical hyperostosis and an increase in the size and number of osteoblasts was noted. When chicks were treated with NaF after hatch and examined at three weeks of age, increased osteoid, cortical thinning, and an expansion of the medullary canal was noted microscopically. $1,25(OH)_2D_3$ had no effect when administered after hatch.

The effects of administering either NaF or $1,25(OH)_2D_3$ together with a slow-onset MAV-2(0) isolate on the morphology of chick bone at the 18 day-old embryo stage, and at 3 and 6 weeks of age will be discussed.

OP24.5

VISNA VIRUS GENE EXPRESSION.
K. Staskus, E. Lewis, E. Retzel and A. Haase. Department of Microbiology, University of Minnesota, Minneapolis, MN, U.S.A.

Visna virus is the prototypic member of the Lentivirus subfamily of retroviruses, and is characterized by a slowly progressive degenerative disease of sheep which involves the joints, lungs, and central nervous system. The slowness of disease progression is attributed to a restriction of viral gene expression at the level of transcription.

We are investigating the role of viral gene products, including those of a putative regulatory nature by analogy to HIV, in this restriction. Our approach includes the systematic interruption of viral open reading frames (specifically Q and S) by site-specific mutagenesis using synthetic oligonucleotides. The effect of these mutations on productive virus infection will be reported.

OP24.6

THE PROMOTER OF RETICULOENDOTHELIOSIS VIRUS STRAIN T COMPETES FOR TRANSCRIPTIONAL FACTOR(S) NEEDED FOR THE PROMOTER FUNCTION OF ROUS SARCOMA VIRUS AND SIMIAN VIRUS 40.
A. Hirano and T. C. Wong. Department of Microbiology, University of Washington School of Medicine, Seattle, USA.

Reticuloendotheliosis viruses (REVs) and avian leukosis-sarcoma viruses (ALSVs) make up the two distinct families of avian retroviruses. REVs share many characteristics with mammalian retroviruses and have been postulated to be of mammalian origin. The promoter function of REV-T in avian and mammalian cells was examined by DNA-mediated gene transfer and in vivo competition assay against the promoters of Rous sarcoma virus (RSV) and simian virus SV40. Despite the resemblance of REVs to mammalian retroviruses, the promoter function of REV-T was highly specific for avian cells and required soluble transcriptional factor(s) titratable with excess amounts of DNA containing the homologous promoter. At least one of the putative factor(s) required for the REV-T promoter function in chick cells was also titratable with either RSV or SV40 promoter. Conversely, The REV-T promoter effectively titrated out factor(s) necessary for the promoter function of RSV in chick cells, and SV40 in primate cells.

These results suggested that the REV-T promoter could interact with cellular factor(s) required for transcription of RSV and SV40, even though transcription of REV-T displayed a strong bias for avian cells.

OP24.7

SEQUENCE ALTERATIONS IN THE DIRECT REPEAT IN 3'UNTRANSLATED REGION OF AVIAN SARCOMA VIRUS MUTANT LA46 CAUSES A CIS-ACTING TEMPERATURE SENSITIVE RNA PACKAGING DEFECT. A. Hirano and T. C. Wong. Department of Microbiology, University of Washington School of Medicine, Seattle, USA.

Avian defective sarcoma virus PRCII contains extensive deletion in all three protein-coding regions (gag, pol and env). Its replication requires a helper virus providing these proteins in trans. LA46, a mutant of PRCII, contains a unique cis-acting temperature sensitive (ts) defect which cannot be complemented by the helper virus. This ts defect is retained in the cloned LA46 DNA. Genetically engineered recombinant virus carrying the 3'untranslated region of LA46 aquired the ts defect. Time course experiments suggested that early replicative events were normal. The promoter function associated with the LTR was also nondefective. The half-lives of nascent viral RNAs transcribed from LA46 and PRCII were comparable at both permissive and nonpermissive temperature. Temperature upshift had no effect on intracellular level of viral RNA. However, RNA packaged into virions was drastically reduced. Sequence analysis revealed that this unique ts lesion was associated with extensive alterations in the direct repeat in the 3'untranslated region.

These results suggested that a temperature-dependent interaction of sequences in the direct repeat with viral or cellular components might be required for packaging of avian retrovirus genome.

OP24.8

COMPUTER EMULATION OF THIN-SECTION ELECTRON MICROSCOPY PREDICTS A NOVEL NUCLEOCAPSID FOR HUMAN IMMUNODEFICIENCY VIRUS. P.A. Marx*, R.J. Mann*, K.I. Joy§. California Primate Research Center*, Department of Medical Pathology*, Department of Electrical and Computer Engineering§, University of California, Davis, USA.

Human immunodeficiency virus (HIV), the retrovirus etiologically linked to acquired immune deficiency syndrome (AIDS) has been characterized by nucleotide sequencing, T cell tropism, transcription-activating gene products and morphological and genetic relationships to the lentivirus subfamily of retroviruses. Little is known, however, about the structure of the virion. Here we provide evidence for a specific kind of nucleocapsid arrangement for HIV. This nucleocapsid structure was suggested by the finding of polyhedral "windows" in electron micrographs (EM) of individual virions of 3 HIV isolates [lymphadenopathy-associated virus (LAV), human T-cell lymphotropic virus-III (HTLV-III) and AIDS-related virus (ARV)] and was tested by a new application of three-dimensional computer modeling that emulates thin-section electron microscopy. With this technique, about 1000 computer-generated "thin sections" of HIV were compared with electron micrographs of HIV. The computer-emulation procedure predicted that the envelope of the HIV encloses an icosadeltahedral nucleocapsid (80-sided, 32 vertices) and this nucleocapsid contains an inner rod-like core abutted between any 2 of the 20 vertices having hexagonal symmetry.

OP24.9

MILK-BORNE TRANSMISSION OF HUMAN T-LYMPHOTROPIC VIRUS TYPE-I AS THE MAJOR NATURAL PATHWAY OF ENDEMY. S. Hino*, H. Doi§, H. Sugiyama*, K. Yamaguchi*, Y. Tsuji§, T. Miyamoto*. Department of Bacteriology* and Pediatrics§, Nagasaki University School of Medicine, Nagasaki, 852 Japan.

Human T-lymphotropic virus type-I (HTLV-I), a causative agent of adult T-cell leukemia (ATL), is endemic in geographically distinct areas. Clusters of carriers in families and cell-to-cell spread of HTLV-I suggested mother-to-child infection. We found that children born to carrier mothers became carriers by 2 years (25%) and that over 90% mothers of young carriers were carriers. The prevalence of young carriers (1%) was consistent with product of the former and the carrier rate of mothers (4%). Postnatal infection was suggested by the absence of infection markers (IgM antibody or HTLV-I antigen) in cord bloods of over 100 babies born to carrier mothers.

Oral inoculation of fresh cells of human carriers' milk (in total 200 ml) into a common marmoset made her a carrier. Moreover, a retrograde study showed that incidences of HTLV-I carriers in children born to carrier mothers were 0/11 (0%), 3/31 (10%) and 14/37 (38%), when they were fed by compound, mixed and breast milk, respectively. The data suggested that milk-borne transmission is the major natural pathway of HTLV-I endemy. Intervention study to refrain from breast feeding by carrier mothers is in progress. This may lead to break up the endemic circuit of HTLV-I, and to eliminate ATL.

OP24.10

ELECTRON MICROSCOPIC EVIDENCE OF REACTION BETWEEN SERA FROM AIDS PATIENTS AND BOVINE LEUKEMIA VIRUS. P.M.Cereda,G.Filice,E.Romero. Institute of Microbiology and Department of Infectious Diseases, University of Pavia,27100PAVIA,ITALY.

Bovine leukemia virus(BLV)is a retrovirus responsible for some aduit bovine leukemia/limphomas. BLV can be isolated and propagated in cell culture.Petal lamb kidney(FLK)cell line is a persistentiy infected cell line producing BLV particles representing a reliable substrate for virus studies. In this work FLK were used in order to verify some data regarding the immunological reactivity of some human sera against BLV.Previously data,obtained by indirect immunoperoxidase staining technique performed on FLK fixed cells,showed a clear reactivity of some human sera to BLV.The same sera were tested by immuno-electron microscopy and the results obtained confirmed the previously reported data referring a frequent reactivity to BLV in sera obtained from AIDS patients and,more rarely, in sera from patients affected by T-cell leukemia.

OP24.11

MORPHOLOGY OF HIV AND RELATED RETROVIRUSES. E. Palmer and C. Goldsmith, Centers for Disease Control, Special Pathogens Branch, Atlanta, GA, USA.

The acquired immune deficiency syndrome (AIDS) is caused by a retrovirus now being termed Human Immunodeficiency Virus (HIV). HIV is morphologically similar to viruses of the Lentivirinae subfamily of the Retroviridae. Direct and immune EM studies show that it is formed at the plasma membrane without involvement of a cytoplasmic precursor. Mature particles consist of an envelope with surface projections surrounding a cone containing an electron dense, eccentric, bar shaped nucleoid. Negatively stained particles show evenly spaced rod-shaped surface projections about 5 nm in length; these appear fuzzy around thin sectioned particles. HIV is markedly different in morphology from Human T cell Leukemia virus types I and II which are typical type C oncoviruses. Lymphadenopathy AIDS virus type II (LAV II) which causes AIDS in West Africa has the same internal morphology as HIV but the spikes around this virus are more prominent than those around HIV. LAV II is similar in morphology to simian lymphotropic virus type III which causes AIDS in captive macque monkeys and to Human T Cell Lymphotropic virus type IV. We present here a comparison of the morphology of HIV and related retroviruses.

OP24.12

THE TRANS-ACTIVATOR GENE OF EQUINE INFECTIOUS ANEMIA VIRUS (EIAV). L. Sherman[1], A. Gazit[1], A. Yaniv[1], T. Kawakami[2], J.E. Dahlberg[2], S.R. Tronick[2], and S.A.Aaronson[2]. [1]Dept. Human Microbiology, Sackler Med. School, Tel-Aviv Univ., Tel-Aviv 69978, Israel, and [2]Lab. Cellular and Molecular Biol., Nat. Cancer Inst., Bethesda, MD 20892, USA.

Employing the transient expression CAT assay, we demonstrated that EIAV is stimulated both in EIAV infected cells and in cells co-transfected with the EIAV genome, indicating the presence of a trans-acting (tat) gene within the EIAV genome. This trans-activation was reflected by an increase in both mRNA and protein levels. Deletion analysis of EIAV-LTR demonstrated that sequences necessary for trans-activation (TAR) are located within the R-U_5 region, downstream from the mRNA initiation site. By testing for the ability of various deletion mutants of EIAV to stimulate the expression of EIAV-LTR CAT plasmid, the critical trans-activating functional domain of EIAV could be mapped to a region between nucleotides 4474 and 5338. This region was found to bear partial homology with the tatIII gene of the human immunodeficiency virus (HIV). The location of the viral-encoded tat gene, the location of the presumed TAR region, as well as the level of control exerted by tat product, provide further evidence for structural and functional similarities between animal lentiviruses and HIV.

OP25.1

CALCIUM DEPENDENCY OF ROTAVIRUS MORPHOGENESIS.
M.S. Shahrabadi and P.W.K. Lee, Department of Microbiology and Infectious Diseases, Faculty of Medicine, University of Calgary, Canada.

Rotavirus is a major cause of gastroenteritis in children and young animals. The infectious virus consists of segmented double-stranded RNA enclosed in a double-layered capsid. Intracellularly, the viral core is formed in the viroplasm and complete virus assembly occurs on the rough endoplasmic recticulum (RER). Previously we showed that the maturation of infectious bovine rotavirus is blocked in the absence of calcium. To determine the mechanism of calcium involvement in rotavirus morphogenesis, pulse-chase experiments were carried out. Subsequent SDS-PAGE analysis showed that synthesis of the major outer capsid protein (VP7) was unaffected by calcium depletion. However, upon chasing in the absence of calcium, VP7 was found to be degraded. Analysis of viral proteins synthesized in the presence of tunicamycin revealed that glycosylation of VP7 was unaffected in the absence of calcium. These results were confirmed by using ^{3}H-mannose to label infected cells. Although the glycosylated VP7 was unstable in a calcium-deprived environment, its nonglycosylated form was somewhat more stable. Electron microscopic examination of infected cells in calcium-free medium revealed that viral cores did not seem to bud through the RER, and that instead, they appeared as membrane-bound structures.

OP25.2

EPITOPE MAPPING OF INFECTIOUS PANCREATIC NECROSIS VIRUS (IPNV) POLYPEPTIDES USING MONOCLONAL ANTIBODIES (McAb). E. Nagy, P. Dobos. Department of Microbiology, Univ. of Guelph, Guelph, Canada.

A collection of monoclonal antibodies against the Jasper strain of IPNV (a double stranded bisegmented RNA birnavirus of fish) was developed. It was known that there are three virion polypeptides (VP1, VP2 and VP3) and one non structural polypeptide (NS) for IPNV. The specificity of the McAbs was determined by immunoprecipitation of ^{35}S labeled IPNV-infected cell proteins and Western blotting of virion proteins. The McAbs were either VP2 or VP3 specific; none reacted with VP1 or NS. All neutralizing McAbs were VP2 specific, suggesting that VP2 is the surface protein. The different epitopes of VP2 and VP3 were also identified by using competetitive ELISA and competetitive neutralization tests.

A hitherto undetected truncated NS was recovered from virions immunoprecipitated by these McAbs or polyclonal serum.

The polyprotein precursor to the three VP's were also identified in infected cells with the aid of these McAbs.

OP25.3

HOW UNIQUE ARE BIRNAVIRUSES? P. Hudson, M. Morgan, I. Macreadie and A. Azad. CSIRO, Division of Protein Chemistry, Melbourne, Australia.

The genome of Infectious Bursal Disease Virus (IBDV) consists of two segments of double-stranded RNA and is thus classified a Birnavirus. The smaller RNA segment (2.9kb) encodes a single 90kD protein implicated as the dsRNA-dependent RNA polymerase whereas the larger RNA (3.4kb) encodes three proteins; the 37 and 32kD structural antigens and a 28kD NS protein. A library of cDNA's constructed from the IBDV genome has been assembled, enabling most of the viral RNA structure to be determined. The smaller RNA encodes an open reading frame capable of translation to a 97kD protein. We believe this protein to be the first example of a novel class of dsRNA replicases since it has no homology to the known ssRNA replicases.

The larger RNA was found to encode in order the 37, 28 and 32kDa proteins as a single chain precursor which must be subsequently processed by viral or cellular encoded proteases. Since our expression studies in E. coli have implicated the 28kD protein in viral processing the IBDV genome organisation and complexity appears to be similar to the single-stranded picornaviruses. Homology to the large RNA of IPNV, the only other Birnavirus characterized, shows significant homology limited strictly to the structural proteins. There is no homology to other viral genome sequences.

OP25.4

GENETIC DETERMINANTS OF PATHOGENICITY FOR TICKBORNE ORBIVIRUSES. S.R. Moss, C.M. Ayres, D. Carey, P.A.Nuttall NERC Institute of Virology, Mansfield Road, OXFORD OX1 3SR, U.K.

Great Island (GI), Nugget (NUG), Broadhaven (BRD) and Nexford (NEX) viruses are serotypes of the Great Island subgroup, in the Kemerovo serogroup of orbiviruses. They have been isolated from ticks collected in seabird colonies, in different geographical locations.

Intra-cerebral inoculation of 2 day-old mice with either GI or NUG viruses resulted in deaths 2-3 days later, and 6-8 days after inoculation of BRD or NEX viruses. Virus distribution and pathology in mice inoculated with different viruses were compared. In order to deduce the genomic segment(s) involved in pathogenicity for mice, spontaneous temperature sensitive (ts) mutants were isolated. Recombinant (ts) viruses were inoculated into mice. Pathogenetic phenotype of the recombinants was determined by comparison with parental viruses. Phenotypic differences were then compared with the genotypes of the recombinants, in order to deduce the genetic determinant(s) of pathogenicity.

OP25.5

GENETIC INTER-RELATIONSHIPS WITHIN THE KEMEROVO SEROGROUP OF ORBIVIRUSES. S.R. Moss, C.M. Ayres, P.A. Nuttall. NERC Institute of Virology, Mansfield Road, Oxford OX1 3SR, United Kingdom.

The Kemerovo (KEM) serogroup has been divided into 4 subgroups (Kemerovo, Great Island, Chenuda and Wad Medani) on the basis of complement fixation tests. The subgroups reflect the origins of the viruses, e.g. all members of the Great Island subgroup have been isolated from hard tick species associated with seabirds.

Polyacrylamide gel electrophoresis of KEM serogroup viruses reveals 10 segments of double-stranded RNA. To determine their potential for reassortment, temperature sensitive (ts) mutants of representatives of the subgroups were isolated. Progeny resulting from dual infection of Vero cell cultures with different ts mutants were screened for recombinant ts virus. The results reflect the genetic relationships both within and between subgroups of the KEM serogroup.

OP25.6

CHARACTERIZATION AND PURIFICATION OF SUBCORE PARTICLES FROM BLUETONGUE VIRUS-INFECTED CELLS THAT HAVE LOST THE MAJOR GROUP-SPECIFIC ANTIGEN P7. H. Huismans, A.A. van Dijk. Biochemistry Section, Onderstepoort Veterinary Research Institute, South Africa.

A study was made of the fate of parental bluetongue virus (BTV) in infected cells. Within the first hour after infection, the BTV particles are converted to core particles with the loss of major capsid polypeptides P2 and P5. From about 6 hr after infection a second uncoating event is observed in which the 470S core particles are converted to 390S subcore particles. These particles are morphologically strikingly different from core particles and have a skeleton-like structure with a hexagonal profile and a side to side diameter of 40 nm. These subcore particles contain only one major structural protein, P3, and three minor proteins P1, P4 and P6. They do, however, contain all 10 double-stranded RNA segments. The results suggest that the characteristic capsomeres on the surface of core particles are mainly composed of P7, the soluble group-specific antigen of BTV. The subcore particles are only stable at very low salt concentrations. Under these conditions no transcriptase activity can be demonstrated.

OP25.7

STRUCTURAL ORGANIZATION OF BLUETONGUE VIRUS. M.J. Grubman. Department of Molecular Biology, USDA, ARS, NAA, Plum Island Animal Disease, Greenport, NY, USA.

Bluetongue virus (BTV) contains 10 segments of double-stranded RNA and 7 structural proteins, 2 of which are present in a diffuse outer layer. Cleavage or removal of 1 or both of these surface proteins converts the virus into subviral particles (SVPs) and cores, respectively. We are interested in the organization of the structural proteins within the virus particle and are examining virus, SVPs and cores by a number of criteria to address this question.

Radioimmune binding assays (RIAs) with a battery of monoclonal antibodies (MAbs) have been used to examine the surface structure of virus, SVPs and cores. MAbs which bind virus particles have been further tested in plaque reduction neutralization assays. Two MAbs which have a relatively high neutralization titer bind virus particles but not SVPs in an RIA. The SVPs used in this assay still retain fragments of VP3, the type-specific surface protein, after sucrose gradient centrifugation. A number of MAbs which recognise VP7 bind virus, SVPs and cores, but do not neutralize. Although previous results from surface labeling studies have suggested that VP7 is an internal core protein, it appears that some regions of VP7 may be exposed on the virus surface. Additional studies with monospecific antisera and MAbs are in progress.

OP25.8

QUANTITATIVE ANALYSIS OF ROTAVIRUS RNA TRANSCRIPTION AND REPLICATION. M. A. Johnson, M. A. McCrae. Department of Biological Sciences, University of Warwick, Coventry, West Midlands, CV4 7AL.

A system has been developed which allows the quantitation of the synthesis of both strands of the 11 Rotavirus genes during a synchronous infection of tissue culture cells.

Cloned copies of the 11 genes of the UKtc Bovine Rotavirus have been sub-cloned into the polylinker region of the pGem 1 and pGem 2 transcription vectors. Using the T7 and Sp6 promotors flanking this region, high specific activity RNA probes to either strand of the gene have been generated and used in Solution Hybridisations to quantitate the transcription and replication of each gene.

Emphasis has been placed on the transcription of the genes, and its quantitative and temporal relationship to the levels of proteins produced during infection. Replication of the genes has also been studied. Comparison of the molar amounts of each individual gene transcribed and replicated is now possible.

OP25.9

MOLECULAR CLONING OF BLUETONGUE VIRUS TYPE 17. S.Samal, M. J. Grubman. Molecular Biology Laboratory, Plum Island Animal Disease Center, USDA-ARS,Greenport, NY, USA.

The double-stranded RNAs of bluetongue virus (BTV) serotype 17 have been cloned in pUC 18 plasmid using two different strategies. One hundred twenty five clones were produced using polyadenylated denatured double-stranded genomic RNA, followed by cDNA synthesis with reverse transcriptase and oligodeoxythymidylic acid primer. Clones were found to contain sequences of BTV RNA by hybridization to total genomic RNA. Assignment of each clone to the genome segment of its origin is proceeding.

The second strategy involved denaturation of individual BTV genomic segments and use of a random primer to generate cDNA. Forty clones were derived from BTV segment 4. The origin of inserts of all clones were confirmed by hybridization to a probe prepared from BTV segment 4. The size and sequence of these clones will be examined.

OP25.10

LOCALIZATION OF THE NONSTRUCTURAL PROTEIN NS1 IN BLUETONGUE VIRUS INFECTED CELLS AND ITS PRESENCE IN VIRUS PARTICLES. B. Eaton, A. Hyatt, J. White. Australian Animal Health Laboratory, CSIRO, P.O. Box 24, Geelong, Victoria, Australia.

The nonstructural protein NS1 is the major protein synthesized in bluetongue virus (BTV)-infected cells. It is the sole constituent of tubular structures which are found associated with intermediate filaments. A panel of monoclonal antibodies (MAb) against NS1 has been used to locate the protein in fixed cells and cytoskeletons using immunofluorescent and immunogold labeling procedures. Cytoskeletons for electron microscopy were prepared from BTV-infected cells grown on parlodion coated gold grids. The results showed that while some anti-NS1 MAb reacted with tubules others reacted both with cytoskeleton-associated virus-like particles and with virus particles adsorbed to the grid substrate outside the infected cell. Subviral particles were isolated from BTV-infected cells by velocity sedimentation in sucrose and by equilibrium sedimentation in CsCl density gradients. The major particle had a buoyant density of 1.31-1.33 g cm^{-3}, sedimented heterogeneously in sucrose gradients, contained double and single stranded viral RNA, protein NS1, the BTV core proteins and small amounts of the outer coat proteins VP2 and VP5. The location of this complex in cytoplasmic and cytoskeletal fractions of infected cells and its role in virus replication will be discussed.

OP25.11

CHARACTERIZATION OF A NOVEL AVIAN REOVIRUS PROTEIN FOUND DURING INFECTION. F. J. Sterner[1,2], T. J. Schnitzer[2], D. D. Huang[1,2]. [1]Department of Immunology/ Microbiology, [2]Department of Internal Medicine. Rush-Presbyterian-St. Luke's Medical Center, Chicago, USA.

A novel protein, MB_1, having a molecular weight of 58K is found in cells infected with avian reovirus. The protein is not easily immunoprecipitated, but is easily detected after Western blotting and immunostaining. The MB_1 protein is not a major viral protein; it is made in small amounts during infection. The protein appears to be non-structural as it is not found in purified virions. The protein is not the equivalent of the mammalian reovirus ϕ , a cleavage product of μ_{1C}. MB_1 is virally coded. Its synthesis can be followed in infected cell lysates late in infection. It is first detectable around 14-16 hours post infection at a time when virus infection has entered into its logarithmic phase of growth.

OP25.12

HIGH LEVEL OF EXPRESSION IN E. Coli OF THE NON STRUCTURAL ROTAVIRAL PROTEIN NCVP2

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To further study the role of the non structural rotaviral protein (NCVP2) encoded by the gene 5 of the bovine RF rotavirus strain, we needed a high amount of this protein. For this purpose we cloned the complete coding sequence of the gene 5 in an inducible procaryotic expression vector PKK 233-2. Upon induction we obtained a very high level expression of a protein having in SDS-PAGE the same MW 54 Kd as the native NCVP2. This product was stable in E. Coli and allowed us to purify to obtain specific antisera. These antisera will permit to study the role for NCVP2 during the rotaviral replication. The protein obtained could also allow to detect a potential enzymatic activity associated with NCVP2. The properties of recombinant NCVP2 will be presented.

OP25.13

EFFECT OF CARBOHYDRATE ON THE INFECTIVITY OF A HARD CLAM VIRUS-CV-1. Y. L. Hsu, R. H. Lin, S. T. Lin, P. F. Su. Institute of Zoology, Academia Sinica, Taipei, Taiwan

The virus isolated from the gill of hard clam Meretrix lusoria was designated as CV-1(Chiayi, no. 1). From the EM observation, and resistant to organic solvent, the virus is an unenveloped icosahedron with a diameter of 52 nm. Both FUDR and actinomycin D do not inhibit the virus multiplication, therefore the virus genome is RNA. With two segments of RNA and 4 viral polypeptides in the early infected stage, CV-1 belongs to Birnaviridae, an IPNV-like virus.

^{3}H-Gln labelling experiment showed VP1,VP2, and VP3 are glycoproteins in CV-1. ^{32}P-labelling also showed these proteins are phosphoproteins. We have studied the effect of tunicamycin(TM), an antibiotic which prevents the N-linked glycosylation of nascent proteins, on the replication of CV-1. In the presence of TM, the infectivity of CV-1 was dramatically decreased 10^{4} fold, and the nonglycosylated proteins were synthesized.

OP25.14

COMPLETE NUCLEOTIDE SEQUENCE OF ROTAVIRUS SA11 GENE 4. S.López, C.F. Arias. Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, Mexico City 04510, México.

RNA segment 4 of SA11 rotavirus codes for VP3, the protein responsible for trypsin enhancement of viral infectivity. The nucleotide sequences corresponding to the ends of this gene have been determined. This information together with the previously reported sequence (López et al. Virology 144, 11-19 (1985)) indicates that the complete gene is 2362 nucleotides long, with 5'- and 3'-noncoding sequences of 9 and 25 nucleotides, respectively.

The encoded polypeptide, VP3, has a molecular weight of 86,733 daltons. VP5 and VP8 the trypsin cleavage products of VP3 have a molecular weight of 59,964 and 26,787 daltons, respectively. Interestingly, the deduced amino-terminal amino acid sequence of VP3 does not have the characteristics of a signal sequence. Possible implications of this observation on the morphogenesis of rotaviruses will be discussed.

OP25.15

SYNTHESIS OF REOVIRUS PROTEIN σ1 IN VACCINIA VIRUS EXPRESSION SYSTEM. A. Banerjea, K. Brechling, C. Ray, D. Pickup and W.K. Joklik. Department of Microbiology and Immunology, Duke University Medical Center, Durham, NC, USA

The S1 gene of reovirus (serotype III) encodes the 45 kDa σ1 protein. In order to synthesize this gene product, a cloned DNA copy of the S1 gene was placed into an expression/insertion vector (p1200) that enabled it to be inserted into the genome of vaccinia virus (VV). In the VV-recombinant, the transcription of the S1 gene is under the control of regulatory elements derived from those controlling the transcription of a gene that encodes the major protein component of the cowpox virus A-type inclusions. Immuno dot-blot analysis (avidin-biotin method), using polyclonal antiserum against the proteins of reovirus (serotype III), was used to identify VV-recombinants that expressed the S1 gene. Western blot analyses were used to confirm that the σ1 product of the cloned S1 gene had the same electrophoretic mobility as the σ1 protein of reovirus. Preliminary evidence indicates that σ1 protein is made to about 1% of the total cell protein (∿ 1 mg per liter). Efforts are underway to purify and characterize the protein to study its receptor binding properties.

OP25.16

DISCRIMINATION AMONG REOVIRUS MESSENGER RNAs BY EUKARYOTIC INITIATION FACTORS 4F PLUS 4A: THE ROLES OF CAP ACCESSIBILITY AND 5' PROXIMAL SECONDARY STRUCTURE. T.G. Lawson, M.H. Cladaras, B.K. Ray, K.A. Lee, R.D. Abramson, W.C. Merrick, and R.E. Thach. Dept. of Biology, Washington Univ., St. Louis, MO 63130; Dept. of Biochemistry, Sch. of Med., Case Western Reserve Univ., Cleveland, OH 44106; USA

The mechanisms by which reovirus mRNAs are differentially selected for translation during the cap recognition process have been investigated. Several quantitative experimental techniques have been applied to this question: a) the rates of reaction of different mRNAs with tobacco acid pyrophosphatase, b) the extent of crosslinking of different mRNAs to initiation factors in the presence and absence of ATP, and c) the degree of sensitivity of different mRNAs to ribonuclease action. The effects of ionic strength on all of these reactions has also been investigated. We conclude from these studies that, under ordinary ionic conditions, the steric accessibility of the m^{7}G "cap" moiety of a reovirus mRNA is a major determinant of its translation rate both in vitro and in vivo. However, when the ionic strength is increased to supra-normal levels, an additional feature, namely the amount of secondary structure formed by sequences proximal to the cap, can become rate-limiting for most, but not all, of the reovirus mRNAs. (Supported by NIH and NSF.)

OP25.17

CLONING AND EXPRESSION OF THE GENE CODING FOR THE MAJOR OUTER CAPSID PROTEIN OF SA-11 ROTAVIRUS IN A BACULOVIRUS EXPRESSION SYSTEM. Thomas P. McGonigal, Mary Turon, Sharon Kister, Kathy Komar-Hartnett and Robert E. Smith, Department of Corporate Molecular Biology, Abbott Laboratories, North Chicago, IL 60064.

The major outer capsid protein (VP7) of rotavirus has been identified as the principal antigen involved in the neutralization of virus infectivity. We have cloned and sequenced the gene (gene 9) coding for VP7 from SA-11 rotavirus to develop reagents which may prove useful for investigating rotavirus serotype specificity. Gene 9 has been expressed in a baculovirus-insect cell expression system in our laboratory resulting in the synthesis of a polypeptide with an apparent molecular weight of 34 kilodaltons, consistent with a native form of VP7 observed in virus-infected cells. The protein was found to be predominantly cell-associated with a significant fraction located within the cell nuclei. The recombinant VP7 is antigenic by immunoprecipitation with neutralizing guinea pig anti-SA-11 polyclonal serum and elicits an immune response in guinea pigs as measured by ELISA. The recombinant VP7 did not compete effectively with neutralizing guinea pig serum derived against SA-11 virus in a fluorescent focus assay. The ability of antisera directed against recombinant VP7 protein to neutralize rotavirus infectivity is being evaluated.

OP25.18

MOLECULAR EPIDEMIOLOGY OF ROTAVIRUS INFECTION IN BULGARIA. D.H. Dimitrov, L.M. Shindarov, S.M. Rangelova, B. Chakov. Department of Virology, Institute of Infectious and Parasitic Diseases, Sofia, Bulgaria.

The molecular epidemiology of rotavirus infection was studied in children with gastroenteritis in Sofia, Bulgaria during 1981-1986 and in the city of Shumen during 1984-1986. Altogether in Sofia were analysed 211 fecal samples from hospitalised infants and rotaviruses with eight different electropherotypes were found in the 132 positive samples. In Shumen were tested 131 samples and 103 were positive. In this city in 1984 an outbreak of rotavirus gastroenteritis was caused entirely by rotaviruses with "short" electropherotypes. During 1985 predominated viruses with "short" electropherotypes and there were only two cases in which viruses with "long" electropherotypes were found and one case with mixed infection in which viruses with "short" and "long" electropherotypes were found. On this ground a cycling effect was predicted and in 1986 the rotavirus infection in Shumen was caused predominantly by viruses with "long" electropherotypes identical to the ones which first appeared in 1985, and there were only two cases in which "short" electropherotypes were found.

OP25.19

ABSTRACT WITHDRAWN

OP25.20

SUBTYPING OF SEROTYPE 4 HUMAN ROTAVIRUS STRAINS BY SOLID-PHASE IMMUNE ELECTRON MICROSCOPY USING MONOCLONAL ANTIBODIES. G. Gerna, N. Passarani, A. Sarasini, A. Di Matteo.
Virus Lab , Inst. Infectious Diseases, University of Pavia, 27100 Pavia, Italy.

Two major neutralization (Nt) epitopes were identified by Nt monoclonal antibodies (MAbs) to VP7 of serotype 4 human rotavirus (HRV) strains. Using the solid-phase immune electron microscopy technique with protein A and two Nt MAbs directed to either epitope, all HRV strains examined and collected from different countries all over the world, were classified into two subtypes, represented at a variable proportion. These findings confirm our previous preliminary data (Gerna et al., J. Infect. Dis. 152: 1143-1151, 1985) and suggest that a more detailed analysis of epitopes of VP7 of HRV serotypes might help in the preparation of rotavirus vaccines and in the evaluation of their protective activity. Partially supported by WHO contract HQ/86/075264 and by Reg. Lombardia, Ric. Finalizzata,project no. 875.

OP25.21

INFLUENCE OF TRANSCRIPTION ON AVIAN REOVIRUS PATHOGENICITY. D. D. Huang[1,2] and T. J. Schnitzer[2]. [1]Department of Immunology/Microbiology and [2]Internal Medicine. Rush-Presbyterian-St. Luke's Medical Center, Chicago, USA.

The infection of two avian reovirus pathotypes in chick embryo fibroblasts was characterized. In vivo, pathotype 1733 is highly invasive and lethal and causes a persistent infection which generates arthritis. Pathotype 2177 is avirulent, non-lethal and non-invasive. In vitro both strains are able to yield equivalent titers of virus over a 48 hour period, but the infection with 2177 is much slower. There is a lag during the first 16 hours in replication in which 2177 produces a titer two logs lower than that of 1733. Both strains are able to attach and penetrate host cells with equal efficiency. A comparison of protein synthesis of the field strains show that individual proteins of 2177 are made more slowly than those of 1733. Neither virus inhibits host cell metabolism. A comparison of the early synthesis of mRNA of the two strains shows that strain 1733 is able to synthesize mRNA within the first 2 hours of infection which steadily increases up through 8 hours post infection. Synthesis of 2177 mRNA is barely detectable during that period of time. The data indicate that the lack of pathogenicity of 2177 may be strongly influenced by events occurring during early transcription of viral mRNA.

OP25.22

Molecular Cloning of the Segmented Double-Stranded RNA Genome of Rice Dwarf Virus. Y.Minobe[1], S.Kashiwazaki[2],H.Mori[3] and T.Omura[2]. National Institute of Agrobiological Resources[1], National Agriculture Reseach Center[2],Tsukuba Science City, Japan. Nagoya University[3],Nagoya,Japan.

Rice dwarf virus(RDV),a member of the Phytoreovirus in plant reovirus group, possesses a genome consisting of 12 ds RNA segments and replicates both in monocotyledonous plants and in a few species of leafhopper vectors. The ds RNA genome of RDV was converted to ds DNA and cloned into plasmid pBR322. The resulting transformants were screened by the dot hybridazation method using individual RDV genome RNA segments labeled with ^{32}P-pCp as probe.The size of the cDNA inserts was estimated by comparing the electrophoretic mobility of their PstI cleavage fragments.

Multiple apparent full-length copies of 8 of 12 genome segments were identified.The sequence of cloned genome segments S12,the smallest of the genome segments and S11 were determined.The conserved common terminal sequence was found in both cDNAs of S11 and S12. Complete nucleotide sequence of the two segments will be presented and the predicted products of their genes will be discussed.

OP25.23

Isolation and Comparative Characterization of a Virulent IBDV Strain. Maurice C. Kemp, Anthony U. Chinnah, Samuel E. Glass, Ellen W. Collisson. Dept. of Microbiology and Parasitology, Texas A&M Univerisity, College Station, Texas, U.S.A.

Infectious bursal disease virus (IBDV) is a naked icosahedral bisegmented double stranded RNA virus. In vivo the virus replicates preferentially in proliferating lymphoid cells in the bursa of Fabricius, leading to rapid destruction of the lymphoid follicles and subsequent immunosuppression of the infected host.

The genomes of four IBDV strains varying in their ability to induce pathology were analyzed by gel electrophoresis. Consistent differences in the electrophorectic mobility of the genomic segments were observed. A virulent strain of IBDV (TX-86) was isolated from flocks of infected chickens that exhibited a high mortality. This isolate was compared to vaccine strains and both genomic segments consistantly exhibited lower molecular weights than those of the three other strains.

Comparative immunological analyses further differentiated the TX-86 strain from currently used vaccine strains. ELISA studies showed minimal cross-reactivity between the TX-86 and the vaccine strains whereas cross-reactivity between the vaccine strains were observed. In addition, immunoblot studies showed strain specificity using polyclonal homologous chicken serum. Variation in processing of the structural polypeptides was also noted.

OP25.24

Polyclonal rotavirus-specific cytotoxic T cells cross-react with syngeneic target cells infected with several rotavirus serotypes. Paul A. Offit, Karla I. Dudzik and Harry B. Greenberg. Department of Medical Microbiology, The Stanford University School of Medicine, Stanford, CA., U.S.A.

Rotavirus-induced gastroenteritis is an important cause of disease and death in many areas of the world. The worldwide impact of these viruses has excited interest in disease prevention by vaccine. Large-scale clinical trials of infants orally inoculated with the bovine strain NCDV demonstrated protection against severe gastroenteritis caused by serotypically distinct natural challenge strains. The immunologic basis of this 'heterotypic' protection is unclear.

We investigated the importance of cytotoxic T cells (CTLs) in protection against reinfection with rotavirus strains heterotypic to the immunizing virus. Splenic lymphocytes from adult, C57 B6 mice orally inoculated with NCDV (serotype 6) were stimulated in vitro with NCDV. CTLs lysed NCDV-infected syngeneic primary suckling mouse kidney cells (SMKCs) as compared with mock-infected SMKCs. In addition, CTLs from NCDV-inoculated mice lysed SMKCs infected with the porcine strain OSU (serotype 5), the simian strain RRV (serotype 3), or the simian strain SA-11 (serotype 3). Conversely, CTLs from mice orally inoculated with SA-11 or RRV lysed SA-11-, RRV- or NCDV-infected SMKCs. We are currently investigating the structural specificities of this heterotypic response as well as the importance of these cross-reactive CTLs in passively protecting suckling mice against challenge. We conclude that adult mice orally inoculated with rotaviruses develop a CTL response which is not restricted by serotype. These findings may in part explain the immunologic basis of 'heterotypic' protection against challenge.

OP25.25

Ø6 NUCLEOCAPSID STRUCTURE

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Ø6 is an enveloped dsRNA bacteriophage of Pseudomonas syringae. Its nucleocapsid contains, in addition to the three RNA segments, five proteins. Three of these, P1(85 K), P2(75 K) and P4(35 K), are necessary for the viral RNA polymerase activity. P8(16 K) forms the surface of the NC, the function of P7(17 K) is unknown.

Nucleocapsids of Ø6 were isolated with a Triton X-114 phase separation procedure. The NCs were treated with protein denaturing and chelating agents. The disruption products were analyzed with rate zonal sucrose gradient centrifugation, SDS-PAGE and EM. Experiments with cleavable cross-linkers were also carried out. The cross-linking products were analyzed with 2-dimensional SDS-PAGE.

The NC surface protein(P8) was released from the particles by EDTA as monomers, although it formed multimeric complexes when cross-linked. The NC was shown to contain a cage-like skeleton consisting of P1 only. Disruption of the NC with urea resulted in complexes of P1 with P2 and P4. P1 and P4 were also cross-linked to each other and the RNA.

OP26.1

CF16, A FILAMENTOUS TEMPERATE PHAGE OF XANTHOMONAS CAMPESTRIS PV. CITRI. W. C. Wu, Graduate Institute of Plant Pathology, National Chung Hsing University, Taichung, Taiwan 40227,

Phage Cf16 is filamentous, capable of lysogenizing Xanthomonas campestris pv. citri XW47 by integrating its prophage into the host chromosome and inducing mutation of cysteine synthesis in some of the lysogenized host cells (Wu et al., 1985). Phage Cf16 was characterized further to understand its biological properties. It produced small, irregular, turbid plaques on strain XW47. It was inactivated completely when heated at 80°C for 10 minutes, irradiated under 15-W UV light at the distance of 60 cm for 135 seconds or exposed to organic solvents such as acetone, chloroform, ethyl alcohol, ether, n-butyl alcohol and xylene. It was stable in nutrient broth and distilled water, but not in 50 mM solution of ammonium sulfate, ferrous sulfate, lead acetate, potassium chloride, dibasic potassium phosphate, phosphate buffer, tris buffer, sodium chloride, sodium citrate, calcium chloride, magnessium chloride or magnessium sulfate. Phage Cf16 was adsorbed to strain XW47 at the maximum rate of 78% in 60 minutes. Its latent period was 90 minutes, growth step was completed in 120 minutes and burt size was 8. This phage infected 10 out of 72 strains of X. campestris pv. citri, but not other strains and other species of plant or animal pathogenic bacteria tested. Phage Cf16, when added at the multiplicity of infection of 5, completely lysogenized cells of strain XW47 24 hours after incubation.

OP26.2

THE ISOLATION OF THE BACTERIOPHAGES OF THREE BACTERIAL AGENTS ON TOMATOES AND THEIR USES FOR THE DETECTION. S.Erkan, H.Saygili. Department of Plant Protection, Faculty of Agriculture, University of Ege, Izmir, Turkey 35100.

The main tomato production districts in the western and southern parts of Turkey were surveyed at the stages of seedling and maturation of fruits and collected the diseased plant materials and the soil samples.

The bacteriophages or phages for 3 bacterial agents infecting tomatoes were individually isolated from 28 diseased plant specimens and 72 soil samples. It was found that many phage plaques were existent in 15 samples for P.s.pv.tomato, 8 for C.m.pv.michiganense and 17 for X.c.pv.vesicatoria while there were a few plaques in some samples or no lysis in about 60 ones. Owing to the phages from the diseased plant materials and the soil samples had variations in the plaque morphology, they were purified until the homogenous plaques were appeared. When the phages with homogenous plaques were tested against these 3 bacteria, 9 phages for P.s.pv.tomato, 5 for C.m.pv. michiganense and 11 for X.c.pv.vesicatoria demonstrated a high degree of specifity.

Furthermore, the specific phages were tested against 57 isolates of some plant pathogenic bacteria and 5 specific phages for C.m.pv.michiganense were used in the detection of this bacterium in the infected tomato seeds.

OP26.3

THE STATE OF PHAGE TAXONOMY. H.-W. Ackermann. Department of Microbiology, Faculty of Medicine, Laval University, Quebec, Canada.

At least 2,900 phages have been studied by electron microscopy. Over 2,750 of them (96%) are tailed, belonging to 3 families named Myo-, Sipho-, and Podoviridae (about 800, 1,500, and 450 members). The other phages are cubic, filamentous, or pleomorphic. They belong to 7 small, fundamentally different families called Cortico-, Cysto-, Micro-, Levi-, Ino-, Plasma-, and Tectiviridae. Except for the Inoviridae, which include the genera Inovirus and Plectrovirus, these families are monogeneric. A new group of enveloped, rod-shaped phages with ds-DNA has recently been described in archaebacteria. The distribution of phage groups reflects bacterial phylogenetic relationships and suggests that tailed phages are extremely old in geological terms.

About 2,650 phages of actinomycetes, bacilli, clostridia, cyano- and enterobacteria, Gram-positive cocci, mycoplasmas, pseudomonads, rhizobia, vibrios, and other important bacteria have been surveyed. About 1,400 phages (45%) can be categorized into some 240 low-level taxonomic units or species. Most others, often tailed phages of the Siphoviridae family, are too poorly known for classification. Genus definition in tailed phages is difficult. Phage taxonomy often depends on morphology; more data on DNA-DNA homology and serological relationships are urgently needed.

OP26.4

ISOLATION OF BACTERIOPHAGES FROM DIFFERENT AQUATIC ECOSYSTEMS. K. Padmakumar and K. Ayyakkannu. Centre of Advanced Study in Marine Biology, Annamalai University, Porto Novo-608 502, Tamil Nadu, India.

Bacteriophages were isolated by direct and indirect isolation method from water Samples of Vellar and Coleroon estuaries, Killai backwater and Pichavaram mangrove of Southeast coast of India. Totally 728 bacterial isolates were tested for phage-host systems. From this 192 strains of bacteriophages were isolated using 449 phage sensitive bacterial strains. Phage-host cross-reaction test was performed to ascertain the lytic and inhibition responses between bacteria and phage lysates. The total heterotrophic bacterial population ranged from 6.2×10^3 to 4.1×10^4 CFU/ml. Effect of salinity on phage-host systems were studied.

OP27.1

NEUROPATHOGENICITY OF MUTANT STRAINS OF MOUSE HEPATITIS VIRUS, JHM. N. Goto, S. Makino, K. Fujiwara. Department of Veterinary Pathology, Faculty of Agriculture, Yamaguchi University, Yamaguchi., University of Tokyo, Tokyo, Japan.

Neuropathogenicity of two plaque purified mutant strains of mouse hepatitis virus, JHM-1a and JHM-2c, from DBT cells persistently infected with JHM virus was studied in mice. After intracerebral inoculation of 10^4 PFU of JHM-1a into 4-week-old ICR mice 30% died within 7 days showing viral growth equivalent to that of JHM virus while all mice JHM-2c infected survived with low-titered virus in the brain. At early stage of JHM-1a infection mice showed slight to mild encephalomyelitis less extensive than in case of JHM virus infection and viral antigen was detectable in neurons and oligodendrocytes. Granular cytoplasmic inclusions were detected in oligodendrocytes with dissociation of myelin lamellae of neighbouring axons. Occasional fusion of infected oligodendrocytes and neurons were seen.

After infection with JHM-2c glial cells rather than neurons were affected without significant inflammatory responce. Cytoplasmic inclusions were also detected.

At 4 weeks postinoculation with both mutant viruses viral antigen or virions were detected within oligodendrocytes and demyelination was produced.

OP27.2

CORONAVIRUS IBV: N GENE SEQUENCE COMPARISONS. D. Cavanagh, P. J. Davis. AFRC Institute for Animal Disease Research, Houghton Laboratory, Huntingdon, United Kingdom.

Using virion RNA and reverse transcriptase we have sequenced the first 140 or so 5' terminal bases of the N glycoprotein gene open reading frame (ORF); these code for the 20 or so residues of the amino terminal hydrophilic sequence and the first of the hydrophobic, presumed membrane-spanning sequences. In addition we have sequenced approximately 100 bases in the non-coding region immediately upstream from the ORF. More than 20 strains belonging to the Massachusetts, D212, D3128, D3896, D207 and some other serotypes have been analysed in order to answer the following questions. (1) Do strains with N of 27K or 30K mol.wt. have 1 and 2 glycans respectively? (2) Do simple amino acid substitutions account for the loss/gain of a glycosylation site? (3) Is the transcription-related homology sequence CTTAACAA conserved among IBV serotypes? (4) Is the location of this site, with respect to the start of the ORF, conserved among IBV serotypes? (5) Might sequencing of part of the N gene help to explain the evolution and epidemiology of IBV?

Currently our data indicate that the answers to these questions are (1) yes, (2) no, (3) yes, (4) no and (5) yes.

OP27.3

CHARACTERIZATION OF EPITOPES ON BOVINE CORONAVIRUS GLYCOPROTEINS. D. Deregt and L.A. Babiuk. Department of Veterinary Microbiology, University of Saskatchewan, Saskatoon, Canada.

Virus neutralizing monoclonal antibodies to the E2 (gp190/gp100) and E3 (gp124/62) glycoproteins of bovine coronavirus were used in competitive binding assays (CBAs) for topological mapping of epitopes. These assays defined two distinct nonoverlapping epitopes on the E2 glycoprotein. These monoclonal antibodies all immunoprecipitate gp190, gp100, and their intracellular precursor gp170. Both epitopes were found to be dependent upon disulphide bonds for their antigenic integrity and were mapped to a 67k proteolytic fragment of gp100/E2.

Analysis of the E3 glycoprotein by CBAs indicated two distinct antigenic sites plus a third site which overlapped these regions. Other results indicate that one site could be further subdivided. Preliminary data indicates differential reactivity of monoclonal antibodies to E3 precursor proteins, gp59 and gp118.

OP27.4

OCCURRENCE OF A NEW 72K PROTEIN IN NON-PATHOGENIC MOUSE HEPATITIS VIRUS TYPE 3 DERIVED FROM PERSISTENTLY INFECTED LYMPHOID CELLS. L.Lamontagne, M. Rojas. Department of Biological Sciences, University of Quebec at Montreal, Montreal, Canada.

Mouse hepatitis virus type 3 (MHV3) infection induces various types of sensitivity according to mouse strains. A chronic disease, characterized by a progressive neurological disease and immunodeficiency, was associated to viral persistency in brain and lymphoid organs. Persistent MHV3 infections have been induced in vitro in lymphoid cell lines. The disappearance of in vivo pathogenicity occurred when pathogenic (L2-MHV3) was replicated in such cell lines (e.g. YAC-MHV3). In order to determine virus modifications produced during viral persistency, we have tested the ability of the non-pathogenic YAC-MHV3 virus to replicate in lymphoid cells and macrophages and analyzed the intracellular viral proteins by SDS-PAGE followed by immunoblotting using various antisera. The results indicated that the lymphoid cells and macrophages from susceptible or resistant mice were not permissive to non-pathogenic YAC-MHV3 in contrast with cell permissivity to pathogenic L2-MHV3. Immunoblotting analysis of intracellular YAC-MHV3 proteins revealed an additionnal protein of 72K.This protein was detected using antisera prepared against L2-MHV3 suggesting that it was expressed during in vivo infection. Work is in progress to analyze its role in pathogenicity.

OP27.5

GENERATION OF IN VIVO RNA-RNA RECOMBINANTS OF MURINE CORONAVIRUS IN MOUSE BRAIN. James G. Keck, Shinji Makino, Glenn K. Matsushima, John O. Fleming, Stephen A. Stohlman and Michael M.C. Lai
Department of Microbiology University of Southern California Los Angeles, CA, U.S.A. 90033

Our laboratory has previously reported on the high frequency of RNA recombination between different strains of murine coronaviruses mouse hepatitis virus (MHV). These studies have previously been done exclusively in tissue culture. To determine if recombination occurs in the virus's natural host, we devised a protocol for the isolation of potential recombinant viruses from the brain of co-infected mice. We injected mice intracerebrally with an RNA (-) ts mutant of A59, LA9, and a wild type JHM-DL strain of MHV. Two days postinfection the mice were sacrificed and the brains were homogenized. The homogenate was treated with anti-DL neutralizing antibodies. Plaque isolates were picked at 39°C, the nonpermissive temperature for LA9. The resulting isolates were screened for recombination by RNase T1-oligonucleotide fingerprinting. Based on the fingerprint patterns at least two of these isolates are in vivo recombinants. One isolate has a single crossover in the 3' most gene, gene A. The second isolate has a double crossover. These results indicate that RNA recombination could occur in vivo. Thus it could be an important mechanism for the evolution of coronaviruses. Furthermore, this observation has significant implication for the study of viral etiology of multiple sclerosis.

OP27.6

PURIFICATION OF THE SPIKE GLYCOPROTEIN E2 OF MURINE CORONAVIRUS MHV-A59 BY MONOCLONAL ANTIBODY AFFINITY CHROMATOGRAPHY. C. Daniel, M. Trudel, P.J. Talbot. Institut Armand-Frappier, Université du Québec, Laval, Canada.

Murine hepatitis viruses (MHV) provide excellent animal models for the study of virus-induced diseases of the CNS and GI-tract. Previous studies have emphasized the importance of the spike glycoprotein E2 in the biological activities of the virus and its interaction with the immune system. We have generated a panel of monoclonal antibodies (MAbs) to the A59 strain of MHV, one of which was used for purification of E2 by immunoaffinity chromatography.

Fifteen stable cloned hybridoma lines were obtained which secrete virus-specific MAbs. Two MAbs could neutralize virus infectivity in vitro and recognized the E2 dimer and monomer both in radioimmunoprecipitation and immunoblotting. The affinity purified neutralizing MAb 7-10A.13 was coupled to activated Sepharose beads. Virus contained in serum-free medium from infected DBT cells showing extensive cytopathology was concentrated by polyethylene glycol precipitation, dialyzed, solubilized with Nonidet P-40 (NP-40) and reacted with the immobilized MAb. After extensive washing and replacement of NP-40 by octylglucoside, the E2 glycoprotein was eluted. Analysis by SDS-PAGE followed by silver staining confirmed the specificity of the method and the purity of the E2 dimer and monomer.

(Supported by NSERC of Canada).

OP27.7

THE EFFECT OF ALTERED SODIUM ION CONCENTRATIONS ON TRANSLATIONAL REGULATION DURING MOUSE HEPATITIS VIRUS INFECTION.
G. Nacintyre, L. Hizzen, F. Wong, R. Anderson. Department of Microbiology and Infectious Diseases, University of Calgary, Calgary, Alberta, Canada, T2N 4N1.

The inhibition of mouse L-2 fibroblast protein synthesis during mouse hepatitis virus (MHV) infection may be modulated by changes in the intracellular ionic environment, as has been suggested for other virus:cell systems. Membrane permeability to extracellular Na^+ was measured by culturing MHV-infected cells in the presence of [$^{22}Na^+$]. Na^+ influx into MHV-infected cells increased from 4-6h PI, correlating chronologically with the expression of MHV-mediated cell fusion. Cell fusion was blocked by the addition of a monoclonal antibody against the MHV E2 glycoprotein. This addition resulted in a reduction in the normal influx of [$^{22}Na^+$]. MHV-infected cell cultures were labelled with [^{35}S]-methionine in medium supplemented with NaCl at final concentrations ranging from 150mM-350mM. Incorporation of radio-label into proteins decreased with increasing NaCl concentration, while the ratio of viral to cellular protein synthesis remained relatively constant. Alterations of the intracellular Na^+ and K^+ levels using ouabain had little effect on the pattern of viral/cellular protein synthesis. Using monoclonal anti-E2 antibody to inhibit Na^+ influx, we demonstrated normal inhibition of host protein synthesis. We conclude that MHV-induced shut-off of host translation is not mediated by changes in intracellular Na^+ levels.

OP27.8

CORONAVIRUS-INDUCED CARDIOMYOPATHY IN RABBITS.
J.D. Small[1], J. Soukup[2], R.D. Woods[3], T.M. Gambling[2], and R.S. Baric[2]. Nat. Inst. Environ. Health Sci.[1]; Dept. of Parasit. and Lab. Pract., Univ. of N. Carolina at Chapel Hill[2]; and Nat. Ani. Dis. Ctr., ARS, USDA[3].

Rabbit cardiomyopathy is caused by a virus (RbCV) originally isolated in testicular suspensions from rabbits infected with T. pallidium. RbCV-infection produces fever, uveitis, and death characterized by enlarged hearts and pleural effusion (up to 50 mls). Myocarditis is evident and histological lesions are located in the heart. Serum creatine kinase levels are elevated, consistent with the interpretation that these animals died from RbCV-induced heart failure. Other physiological changes include: decreased RBC and platelet counts, elevated nucleated RBC and WBC counts, and increased prothrombin times from 8.1 to over 150 secs. Previous results from our laboratory suggest that the etiologic agent for rabbit cardiomyopathy is a coronavirus antigenically related to the human coronavirus strain 229E. By RIA, RbCV is also related to FIPV, TGEV, and PEDV, suggesting that this virus is a group 1 coronavirus. RbCV and 229E have a bouyant density between 1.19 - 1.20 g/ml and their nucleic acids cross-hybridize with TGEV cDNA probes. Gradient purified RbCV is infectious and appears as a coronavirus particle in the E.M. Cross-protection studies indicate that FIPV and TGEV protect rabbits against RbCV challenge. These data strongly support a coronavirus etiology for the disease.

OP27.9

CORONAVIRUS TGEV : TOWARDS A PHYSICAL MAP OF PEPLOMER GLYCOPROTEIN E2 EPITOPES.
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Reactivity of E2 partial proteolytic fragments was tested against a set of 23 monoclonal antibodies (Mabs) specific of E2 and/or its precursor E'2. Our results confirmed a previous topographical map which defined four main antigenic sites, termed A, B, C and D. (i) The collagenase 28 K fragment was recognized by anti A-B Mabs. (ii) The trypsin 43 K and 46 K fragments were recognized essentially by anti D Mabs. (iii) The chymotrypsin 14 K fragment was recognized by 19 Mabs specific of ABCD and other minor sites. However anti AB Mabs exhibited lower reactivity for the 14 K fragment than for mature E2, suggesting the importance of conformational factors. The chymotrypsin 23 to 27 K fragments were recognized only by four anti E'2 Mabs. Importantly, no additional immunoreactive specificity could be evidenced using polyclonal antibodies.
The influence of deglycosylation upon E2 antigenicity was also studied. (i) Mild periodate oxydation was not found to be a suitable method since it induced a cleavage of polypeptide chain concomitantly to deglycosylation. (ii) Using endoglycosidase H treatment under various conditions, only the E'2 species could be deglycosylated, resulting in a polypeptide with MW 140 K which still retained part of E2 antigen reactivity.

OP27.10

PHOSPHATIDYL-SERINE INHIBITION OF HUMAN OC43 AND BOVINE NCDCV CORONAVIRUSES.
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Human coronavirus OC43 and bovine coronavirus NCDCV(Neonatal calf diarrhea coronavirus) show a strong inhibition of all their biological properties(replication, infectivity and antigenic expressions)both in vivo and in vitro in presence of serum of mammalian origin. Inhibition can be present also in sera free of antibody, as generally occurs in fetal calf serum used for cell culture growth and maintenance. Several studies were carried out for characterization and to determine virus-specific broad-spectrum. Results obtained showed that phosphatidyl-serine is the natural substance, active towards coronavirus OC43 and NCDCV, present in mammalian sera. Inhibiting activity can be removed by treatment of sera with some enzyme like phospholipase C. Removal of phosphatidyl-serine from sera can allow use of sera for virus isolation and cultivation and reliability of serological tests.

OP27.11

CELL-FREE TRANSLATION OF HUMAN CORONAVIRUS 229E mRNAs.
P. Jouvenne[1], C.D. Richardson[2] and P. J. Talbot[1]. [1]Institut Armand-Frappier, Laval, Québec, Canada and [2]Institut de recherche en biotechnologie, Montréal, Québec, Canada.

Human coronaviruses (HCV) are responsible for up to 25% of common colds and have been associated with pneumonia, myocarditis, neurological disease and gastrointestinal disorders. The fact that a large majority of the human population possesses anti-coronaviruses antibodies suggests that either frequent reinfections or viral persistence occur. Nevertheless, our rudimentary knowledge concerning these viruses and the lack of diagnostic tools have not yet allowed a verification of their medical importance.

In order to investigate the synthesis of viral proteins, intracellular viral RNAs were extracted by the guanidinium-isothiocyanate method. Poly A-tailed mRNAs were selected by oligo(dT) chromatography and translated in a rabbit reticulocyte cell-free system. To confirm the viral nature of the translated products, they were precipitated with standard polyclonal antiserum. SDS-PAGE electrophoresis of the immunoprecipitated polypeptides revealed several proteins corresponding to 170, 60, 42, 19 and 13 Kd. By analogy to other coronaviruses, the 170, 60 and 19 Kd proteins could correspond to the dimeric E2, N and E1 structural proteins, respectively. The 42 and 13 Kd polypeptides may be non-structural proteins. Characterization of proteins from purified virions should confirm identification of these polypeptides. With a view to study the molecular biology of HCV 229E, synthesis and cloning of cDNAs from the viral genome are in progress.

(Supported by Medical Research Council of Canada).

OP27.12

MOLECULAR AND ANTIGENIC VARIATIONS IN SEVERAL STRAINS OF AVIAN INFECTIOUS BRONCHITIS VIRUS.
E. Collisson, L.Wang, L.Sneed, M.Kemp, B.Parr, G.Butcher and C.Pixley. Department of Veterinary Microbiology and Parasitology, College of Veterinary Medicine, Texas A&M University, College Station, TX.

Avian infectious bronchitis virus (IBV) is a coronavirus which causes respiratory disease in chickens. The virus also affects the genital tract and kidneys. There is considerable serological variation within this group. The Arkansas serotype is currently widely distributed in the U.S.A. and is serologically distinct from the Mass-41 and Conn strains which are more commonly vaccinated against. An Arkansas strain, designated Ark-TX83, was isolated from the trachea of a chicken in Gonzalas, TX in 1983.

The antigenic and molecular variations of Arkansas serotype strains were studied. Immunoblot analysis of several serotypes, including the Arkansas type, indicated that the Arkansas strains were antigenically distinct from the Aust-T, Mass-41, Grey and Holland-52 strains. The peplomer and nucleocapsid proteins and, only to a minor extent, with the peplomer proteins of the Arkansas viruses. Comparisons were also made between the genomes of Ark-TX and other strains of IBV. Segments of the Ark-TX83 strain have been cloned, sequenced and compared with the published data of the Mass-41 and Beaudette strains.

OP27.13

MOUSE HEPATITIS VIRUS RECEPTOR INTERACTIONS. K.V. Holmes, R.K. Williams, C.S. Stephensen, S. Compton, D.G. Weismiller, C. Cardellichio. Department of Pathology, Uniformed Services University of the Health Sciences, Bethesda, MD 20814

The plasma membrane receptor for the murine coronavirus MHV has been isolated from intestinal brush border membranes of susceptible strains of mice. The receptor is a glycoprotein of about 110K. The MHV receptor activity and antigen are not found on membranes from SJL/J mice which are genetically resistant to infection with MHV. Infectivity of cells in culture can be blocked by polyclonal antibody and by one of 40 monoclonal anti-receptor antibodies. Blocking is specific for MHV, and these sera do not prevent infection of cell lines with human coronavirus 229E. The MHV receptor is detectable on membranes of cells from susceptible strains of mice and on many murine cell lines, but not on human, hamster, rat, chicken or dog cells or cell lines which are not susceptible to MHV infection. These data suggest that the absence of a specific MHV receptor from membranes of SJL/J mice accounts for their genetically determined resistance to infection with MHV. In addition, the receptor appears to determine the species specificity of coronavirus infection.

OP27.14

STUDIES RESULTING FROM THE SEQUENCE OF THE NON-STRUCTURAL PROTEINS OF CORONAVIRUS MHV-A59. P.J. Bredenbeek, W. Luytjes, J.F.H. Noten, B.A.M. van der Zeijst[1], W.J.M. Spaan. Institute of Virology and [1]Section of Bacteriology, Veterinary Faculty, State University Utrecht, Yalelaan 1, 3508 TD Utrecht.

Mouse Hepatitis Virus Strain A59 is one of the best studied members of the Coronaviridae. The intracellular mRNAs form a 3' nested set with sequences extending for different length in the 5' direction. In addition to the sequence homology at the 3' end all MHV RNAs share a common leader sequence. The mRNAs are generated by a discontinuous transcription process.

To analyse the viral proteins and nucleotide sequences involved in the discontinuous transcription process we have cloned the viral genome. Several non-structural viral genes were sequenced and recloned into the bacterial expression plasmid pEX.

B-galactosidase fusion proteins were used to raise antibodies against several MHV-A59 non-structural proteins. The antibodies enable us to study the expression of the genes encoding the non-structural proteins during virus infection and to obtain information about the function and subcellular location of these proteins.

OP27.15

ROLE OF CORONAVIRUS TGEV TRANSMEMBRANE PROTEIN E1 IN INTERFERON INDUCTION. H. Laude, B. Charley, J. Gelfi, B. Delmas, C. La Bonnardière, D. Rasschaert.
INRA, Station de Virologie, Thiverval-Grignon, France.

The IFN response of TGEV infected pig neonates is predominantly of α-type. Peripheral blood lymphocytes, in which the virus do not replicate, appear to be the main contributors to α-IFN synthesis. An assay consisting of non-immune lymphocytes cultured for 8-16h in the presence of infected fixed cells was used to investigate the mechanism of IFN-induction. Two monoclonal antibodies (Mabs) against the protein E1 were shown to block the induction, which was unaffected when other anti-E1 Mabs or anti-peplomer protein Mabs were added. The 2 blocking Mabs recognized partially overlapping epitopes, and were able to strongly neutralize virus infectivity together with complement, thus allowing to select escape mutants. Hundred single or double epitope mutants were selected. All were found to retain their IFN inducing activity, even in the presence of the blocking Mabs.This demonstrates that 1) E1 is specifically involved in commiting lymphocytes to produce IFN. 2) The interferogenic domain of E1 is adjacent to the modified epitopes. Amino acid data predict that E1 possesses a short hydrophilic, putatively exposed, N-terminal segment, which comprises 30 residues, including 4 Cys and 1 Asn-linked sugar chain. We suggest that IFN synthesis is triggered by interaction of this domain of E1 with the lymphocyte surface.

OP28.1

ANTI-IDIOTYPIC MONOCLONAL ANTIBODIES AGAINST NEUTRALIZING ANTI-CANINE PARVOVIRUS MONOCLONAL ANTIBODIES.

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Canine parvovirus (CPV) is a non-enveloped single stranded DNA virus that may cause an enteritis-panleukopenia syndrome in dogs. Monoclonal anti-idiotypic antibodies (MoAb2) were raised against two different neutralizing monoclonal antibodies (MoAb1) which recognize two different epitopes on CPV. 15 MoAb2 reactive with F(ab)2 or Fab fragments of the respective MoAb1 were selected on the basis of the inhibition of MoAb2-MoAb1 binding by CPV. All these MoAb2 recognized private idiotopes exept for one, which recognized a cross-reactive idiotope as demonstrated by the inhibition of Ab2-Ab1 binding by hyperimmune sera to CPV from different species.

These results suggest that this MoAb2 may act as a true internal image of a viral epitope. The ability of these MoAb2 to induce or to modulate an immune response to CPV is precently under investigation.

OP28.2

POLYOMA VIRUS STRUCTURE AT 8 Å RESOLUTION.

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Polyoma is a DNA virus which induces tumors in newly born or immunologically deficient mice. The virus particle consists of a double-stranded DNA in close association with an equal weight of cellular histones. This chromatin core is surrounded by a protein coat composed of 72 capsomeres arranged on a T = 7d icosahedral surface lattice. Each capsomere in turn consists of five copies of the major coat protein termed VP1. This finding is inconsistent with the Caspar and Klug theory which predicts both pentameric and hexameric capsomeres in the structure.

The structure of polyoma virus has been determined to 8 Å resolution using molecular replacement phase extension starting from 22.5 Å resolution. The resultant electron density map shows that the hexavalent pentamers at the local fivefold axes and the pentavalent pentamers at the icosahedral fivefold axes have similar structures. Furthermore, each monomer within the pentamer has two ordered domains, one protruding and the other in the viral shell.

OP28.3

ADENOVIRUS INFECTION OF HUMAN LYMPHOCYTES.

J. Horvath, J. Weber. Department of Microbiology, Faculty of Medicine, University of Sherbrooke, Quebec, Canada.

Group C adenovirus DNA sequences are frequently found in peripheral blood lymphocytes (PBL) of healthy adults. Although sometimes the complete viral genome is present, rescue of infectious virus requires cocultivation with permissive cells (Horvath et al, J. Virol. 59, 189, 1986. We attempted to determine the block in infection of these cells. Adsorption of fluorescent (FITC) virions showed that receptors on lymphocytes were about ten times less than found on permissive HEp2 cells. The uncoating process was slower and DNA synthesis started later, between 24-48 h.p.i. There was no virus assembly in resting lymphocytes but phytohemagglutinin treatment permitted production of about one virus particle per cell after 4 days of infection. We conclude that adenovirus infection of PBL is blocked at multiple steps, thereby permitting the observed persistence of viral DNA in cryptic form.

OP28.4

STRONG ASSOCIATION OF HUMAN PAPILLOMA VIRUS (HPV) 16 WITH FEMALE GENITAL PRECANCERS .

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Cervical,vaginal and vulvar biopsy specimens from 180 patients with an abnormal Papanicolau smear were analyzed for HPV 6,11,16, and 18 by nucleic acid spot hybridization and the results correlated with microscopic findings.Histological analysis was based on the classification used by Crum et al., J Virol 54,675-681,1985.DNA was extracted , spotted on filter, and sequentially probed under stringent conditions (Tm -23°C) with ^{32}P-labelled HPV DNA inserts of HPV plasmids, kindly made available by prof. H.zurHausen, Heidelberg.The results were following:

Type	No	Pathology				
		C	CINK VINK	CIN VIN	Negative	No dg
HPV 6	22	18	3		1	
HPV 11	3	3				
HPV 16	24	7	8	6	1	3
HPV 18	22	14	6		1	1
HPV 6 + 11	2	2				
HPV 11 + 18	1	1				

Strong correlation of HPV 16 with precancers was found. This supports the concept of HPV 16 being associated in the genesis of genital cancers.

OP29.1

CALF VIRUS INFECTIONS IN NORWAY, A SEROSURVEY.

B. Hylleseth, H.J. Larsen, K. Norheim. The Norwegian College of Veterinary Medicine, 0033 OSLO 1, Norway.

In a study of calf diseases in Norway 250 sera were collected from 5 districts. One hundred of the 250 sera were from 1 week old calves (A) and 150 were from calves aged 12-22 weeks (B). These sera were tested for antibodies (ab) to 8 viruses and Chlamydia psittaci as shown below.

		% positive**		District percentages*				
Virus	Test	A	B	1	2	3	4	5
Bov.resp.sync.	NT	17	11	8	4	2	50	2
Bov.v. diar.	NT	25	25	10	18	14	58	26
Bov.rota	NT	100	100	100	100	100	98	100
Parainflu.3	HIT	85	64	56	74	74	88	70
Reo 1	HIT	53	21	36	28	52	44	8
Reo 2	HIT	27	8	28	16	18	10	6
Reo 3	HIT	61	6	32	36	30	26	16
Bov.herp.1	NT	0	0	0	0	0	0	0
Chlam.psitt.	CFT	57	56	64	42	58	64	54

* Based on 50 calves in each district; no virus vaccine used. ** Ab in A-calves are presumably maternal in origin whereas ab in B-calves include both maternally derived and from infections.

Results indicate rather high prevalences for many of the tested viruses. District differences are most pronounced for bovine respir. syncytial & v. diarrhea viruses.

OP29.2

THE ROLE OF INFECTIONS IN NEUROLOGICAL DISORDER. Pamela J. Siegel Randolph and George Howard III. Neurological Referral Center, Boston University Medical Center, Boston, MA 02118 U.S.A.

A study of sixty patients, as to cause of their neurological complaint indicated close to twenty percent were a result of infections spreading to the central nervous system. For this reason, quicker treatment of infections is in order. New antiviral and antibacterial agents should be considered which are more effective in treating infections of the central nervous system. We hope to be able to determine which agents commonly cause damage to the central nervous system in an otherwise ordinary healthy population of adults.

OP29.3

INFLUENZA A H3N2 CHALLENGE IN A CHRONIC CARE HOSPITAL FOLLOWING A 7-YEAR PROGRAM OF ANNUAL INFLUENZA IMMUNIZATION

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The effectiveness of maintaining an annual vaccine program for 7 years (1976-82) in 60% of 545 patients in a chronic care hospital was put to test in 1982-83 by a significant epi-demic challange with wild type influenza A H3N2 subtypes. For years 1-6 vaccines used were inactivated whole virus, which in year 7 was administered to 60% of vaccinees, the oth-er 40% (with previous whole virus vaccination) receiving split virus vaccine. Of 74 respiratory infections, 30 were of A H3N2 etiology (Bangkok/79, Arizona/80, Philippines/82) determined by a combination of virus isolation (LCDC.CDC identified) and serology of acute/convalescent serum pairs. Of the 30, 19 were not vaccinated in 1982, and 11 were, 5 with split vaccine. The attack rate (AR) in the vaccinated was a highly significant 2.4-fold less than in the non-vaccinated, respectively 3.4% vs 8.5% (p 0.006). Vaccine efficacy at 59.6% was well above other reports in equivalent institutions Although only 60% of the patients were vaccinated, Bangkok specific Geometric Mean Titre (GMT) achieved, 67.88, compared to the non-vaccinated of 16.54, reflected a sufficiently high herd immunity to form a resilient core blanketing the overall patient population making it refractive to an extensive influenza outbreak as observed in 1982-83. This supports the policy of annual vaccination in chronic care hospitals.

OP29.4

SEROEPIDEMIOLOGICAL STUDIES OF HTLV-I IN THE PORTUGUESE POPULATION.

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HTLV-I has been associated with adult T-cell Leukemia/ /Lymphoma. The geographical distribution of the virus raised the possibility that it was disseminated by the portuguese from Africa. A seroepidemiological survey of healthy donors was carried out.

For this purpose 6 400 sera samples of men and women were taken in different zones of the country. Groups were made with people having lived in the former portuguese colonies of Africa. Samples from people that has never lived in Africa were included as controls.

Preliminary results, obtained by ELISA, indicate that the prevalence of the virus is low and distributed among all groups.

OP29.5

CRIMEAN-CONGO HEMORRHAGIC FEVER INFECTION IN 15 VERTEBRATE AND 7 TICK SPECIES. A.J. Shepherd and R. Swanepoel. National Institute for Virology, Sandringham 2131, Republic of South Africa.

Fifteen species of domestic and wild vertebrates were infected with Crimean-Congo hemorrhagic fever (CCHF) virus in order to determine the potential role of each in the virus transmission cycle. Low-titered viremia (maximum 4.0 $\log_{10}$ LD_{50}/ml) followed by development of antibody occurred in sheep, calves, hares, guinea fowl and 5 rodent species. Antibody response only was detected in hedgehogs and a further 4 rodent species, but domestic chickens were refractory to the virus. Ticks failed to become infected when fed on viremic sheep and rodents, but the virus was transmitted to ticks which fed on calves and hares. However, transovarial transmission of virus did not occur in the adult ticks which fed on calves.

The virus failed to replicate in 3 argasid tick species inoculated intracoelomically, but multiplied to high titer (maximum 6.3 $\log_{10}LD_{50}$/ml) in 4 ixodid tick species and persisted in the ticks for up to 205 days. Three of the ixodid tick species transmitted CCHF infection to sheep by bite, but transovarial transmission of virus to the progency of infected females was not detected.

OP29.6

DETECTION AND DIFFERENTIATION OF HUMAN ROTAVIRUSES FROM CHILDREN WITH GASTROENTERITIS IN TAIPEI, TAIWAN FROM 1981 to 1985. C.N. Lee, C.L. Kao, C.Y. Lee. Department of Medical Technology and Pediatrics, College of Medicine, National Taiwan University, Taipei, Taiwan

Stool specimens were collected from 340 children with acute gastroenteritis at the National Taiwan University Hospital from July 1981 to May 1985. Rotaviruses were detected by Rotazyme test and confirmed with solid-phase immune electron microscopy. Rotaviruses were found in 75 samples. Forty-nine percent of the positive specimens were distributed in the coldest months of year, i.e.,December and January. Ribonucleic acids were extracted from 43 specimens and analyzed in polyacrylamide gel electrophoresis. There were 4 electropherotypes which were recognized as different from the RNA pattern of laboratory reference strains (Wa,KU, YO,and S2) and the strain previously collected in 1978. Most of the samples from the same epidemic had similar electrophoretic pattern. A sample was found with extra RNA bands that might reflect a mixed infection of two rotaviruses. Subgroup specificity was determined by enzyme-linked immunosorbent assay using monoclonal antibodies with subgroup I and subgroup II specificity. Approximately 30% of the tested rotaviruses belonged to subgroup I, the number of rotaviruses with subgroup II specificity was about twice that of subgroup I.

OP29.7

COMPARATIVE EPIDEMIOLOGY OF MEASLES AND CHICKENPOX DURING INFANCY AND EARLY CHILDHOOD. K.Baba,H.Yabuuchi,K.Tanaka,H.Yoshida,PL Ogra,M.Takahashi.Department of Pediatrics,Osaka University School of Medicine and Children's Hospital of Buffalo,New York.

The frequency and clinical attack rate of outbreaks of chickenpox were compared with those of measles among institutionalized children younger than 3 years,over the period of 36 years since 1950 Osaka,Japan. During the period,2.8±0.6 outbreaks of chickenpox(n=26) occurred every 4 years,the frequency of which was significantly($p<0.005$) higher than that(1.2±0.6) of measles(n=11) in the same population. The overall attack rate of chickenpox was always 100% during each outbreak among children who had no history of the disease,including those younger than 1 years. In contrast,12 to 45% of children younger than 1 year did not develop clinical measles even when the attack rate among those older than 1 year was 100%. None with maternally acquired FAMA(fluorescent antibody to membrane antigen)titer of≥1:16 for measles developed illness during exposure. On the other hand,all with maternal VZV FAMA antibody of 1:2 to 1:16 had clinical chickenpox. These results may explain the epidemiologic difference of the diseases and the widely held question why infants younger than 1 year develop chickenpox postnatally more frequently than measles.

OP29.8

SEVERE HEMORRHAGIC FEVER WITH RENAL SYNDROME IN GREECE: CLINICAL AND EPIDEMIOLOGICAL CHARACTERISTICS, AND ISOLATION OF THE ETIOLOGICAL AGENT. J. W. LeDuc and A. Antoniades. U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD; Aristotelian University of Thessaloniki, Thessaloniki, Greece.

A severe form of hemorrhagic fever with renal syndrome (HFRS) exists in Greece. The disease is characterized by fever, abdominal or back pain, conjunctival injection, renal insufficiency and a mortality rate currently estimated at 10-20%. More than 20 cases of this disease have been diagnosed since we initiated our investigations in 1982. Typical cases are adults, usually male, and generally with a history of rural exposure. Most cases occur in summer or fall months, and rarely during the winter. Small mammal survey results suggest that Apodemus flavicollis may be the host of the virus. A hantavirus was recently isolated from the urine of a critically ill HFRS patient in Greece, which we suspect is the etiological agent of this disease. The patient's disease was characterized by fever, shock, hemorrhagic manifestations, and renal insufficiency. The virus was isolated from urine obtained on the 7th day of illness by cocultivation in Vero E-6 cells. Serological tests found the isolate to be closely related to prototype Hantaan virus, and distinct from Seoul and Puumala viruses, the other known causes of HFRS. Neutralization tests found high antibody titers to the isolate in convalescent sera of previously diagnosed severe HFRS Greek patients.

OP29.9

THE APPLICATION OF THE ANTIBODY DEPENDENT PLAQUE ENHANCEMENT TEST IN SEROEPIDEMIOLOGICAL SURVEYS.
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Dept of Microbiology,Faculty of Medicine,
University of Peradeniya,Peradeniya,Sri-Lanka.

Antibody dependent plaque enhancement (ADPE) of many arboviruses can be demonstrated on the macrophage cell line P388D$_1$.This assay is comparable to immunoassays in sensitivity and is serologically "broad" in reactivity(Peiris&Porterfield 1982 J.gen.Virol 58,291).Inthe course of a seroepidemiological survey,we have tested over 500 sera (of human,cattle,goat, sheep,pig and dog origin) by ADPE to West Nile(WNV);Getah; Sindbis;Batai and Sathuperi viruses in parallel with plaque neutralisation tests(PNT) to WNV;Dengue2(Den2);Japanese Encephalitis(JE);Getah;Sindbis;Chikungunya;Batai and Sathuperi viruses.The results showed that
a) the proportion of sera positive by ADPE is greater than by PNT(eg.Batai ADPE-22%;PNT-11%), even when ADPE to a single virus is compared to a panel of PNT tests to serologically related viruses(eg.WNV ADPE-28%;WNV/JE/Den2 PNT-16%)
b)ADPE tests to one representative virus(WNV) can detect the majority (85%) of sera positive by PNT to a panel of serologicaly related viruses(JE;Den2;WNV).

These results indicate that the ADPE test could be an useful screening test in seroepidemiological surveys.One limitation of this technique is that it is not applicable with avian sera.

OP29.10

HORSE VIRUS INFECTIONS IN NORWAY, A SEROSURVEY.

B. Hylleseth, H.J. Larsen. The Norwegian College of Veterinary Medicine, 0033 OSLO 1; K. Johansen, Bjerke Animal Hospital, 0515 OSLO 5, Norway.

A serosurvey has been carried out on sera collected from 161 adult and 357 young (7-10 months) racehorses. The latter were selected from 6 different districts in order to find possible active infections. These sera were tested for antibodies (ab) to 11 viruses with the following results as percentage seropositives:

Virus	Test	Adult	Young	Virus	Test	Adult	Young
Eq.rhino 1	NT	68	14	Eq.influ.1	HIT	88	1
Reo 1*	HIT	75	27	Eq.influ.2	HIT	81	0
Reo 2*	HIT	46	15	Eq.influ.F	HIT	73	0
Reo 3*	HIT	62	63	Parainflu.3	HIT	0	0
Bov.rota*	NT	76	50	Eq.herp.1	NT	95	10
Eq.arteri.*	NT	39	6				

Eq. = equine; Bov. = bovine; F = Fontainebleau
* Ab first time shown in Norway.

Equine influenza has not been reported in Norway during the last 8 years. The high percentages of seropositive adult horses for the 3 eq. influenzaviruses are presumably due to efficient vaccination. The 8% non-vaccinated young horses positive to eq. influenzav. 1 are probably due to maternal ab since the titers were borderline low. Levels of ab to most of the other viruses investigated in the young horses indicated active infections in several districts.

OP29.11

DURATION OF MATERNAL ANTIBODIES IN CALVES TO FOUR BOVINE VIRUSES AND CHLAMYDIA PSITTACI.

B. Hylleseth, H.J. Larsen, K. Norheim. The Norwegian College of Veterinary Medicine, 0033 OSLO 1, Norway.

In a study of calf diseases in Norway sequential sera were collected from a group of 16 calves aged 1 week to 6 months. These sera were tested for antibodies (ab) to: Bovine respiratory syncytial virus (BRSV), calf rotavirus (ROTAV), parainfluenza type 3 virus (PIV-3), reovirus type 1 (REOV-1) with the following results:

Virus	Test	Titer at 1 week*	Weeks to reach ab low level**
BRSV	NT	1.11	18
ROTAV	NT	1.48	12
PIV-3	HIT	2.26	14
REOV-1	HIT	1.54	4

* Mean $\log_{10}$ ab titer at 1 week of age.
** Age in weeks (interpolated value) to reach ab titer low level of 0,6 for NT, and 1,3 for HIT. None of the calves showed rising ab titers to the 4 viruses.

A parallel test (CFT) for ab to Chlamydia psittaci antigen showed low levels (average titer of 1.09) of maternal ab at 1 week that declined below detection level (0,7) at about 3 weeks of age; a rise in ab indicated active infection in 13 of the 16 calves at 3-6 months of age.

OP29.12

STUDY ON THE CIRCULATION OF ROTAVIRUS, HEPATITIS A VIRUS AND LISTERIA IN SCHOOL-CHILDREN.
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Virological and bacteriological investigations for Rotavirus, Hepatitis A virus (HAV) and Listeria in clinically healthy school-children (7 - 12 years old) were carried out.

1036 faecal samples from 259 children were examined by ELISA method. 29 of them were positive for Rotavirus (11,19 %), 2,7 % were positive for HAV and 3,9 % were registered to have both, Rotavirus and HAV.

12 Listeria strains were isolated (4,63 %), 5(1,9%)children had both, Listeria and Rotavirus. The serovar background of the isolated Listeria strains and their group circulation was determined. An epidemiological inquiry of the studied contingents was carried out.

OP30.1

STEPS IN THE ASSEMBLY OF VESICULAR STOMATITIS VIRUS NUCLEOCAPSIDS. P.J. Dillon and K.C. Gupta. Department of Immunology/Microbiology, Rush Medical College, Chicago, IL, USA.

The process of nucleocapsid assembly of vesicular stomatitis virus (VSV) was studied in infected BHK cells. Nucleocapsids at various stages of assembly were isolated from nuclease treated cytoplasmic and cytoskeletal extracts and separated for size by velocity gradient centrifugation. Nuclease protected viral RNAs were analyzed by denaturing gel electrophoresis. The smallest viral RNA protected was approximately 60 nucleotides in length. Nucleocapsid assembly proceeded in a step-wise fashion with the protection of ~9 additional nucleotides at a time, presumably by successive binding of monomeric N protein. This observation correlates to the reported molecular composition of mature nucleocapsids that there are 1258 copies of N protein to the 11,162 nucleotides in the viral genome giving a ratio of ~9 nucleotides per N protein.

Protein analysis of the isolated nucleocapsids revealed that both N and NS proteins were associated with the smallest viral RNA species, suggesting that both proteins are involved in the initial encapsidation events. The majority of the nucleocapsid population was found to be associated with the cell cytoskeletal framework and protein analysis indicated that a cellular protein (possibly actin) may play a role in nucleocapsid maturation.

OP30.2

GROWTH RESTRICTION OF NEGATIVE STRAND RNA VIRUSES IN A RAT 3Y1 CELL LINE. Harumi Sano and Y. Nagai. Research Institute for Disease Mechanism and Control, Nagoya University School of Medicine, Nagoya 466, Japan.

A rat cell line, 3Y1, was found to be unable to support the growth of several RNA viruses including influenza (A/WSN), Sendai, Newcastle disease and vesicular stomatitis viruses. To elucidate the mechanism of the restriction of the virus growth, we analyzed the virus-cell interactions from various aspects, using a comparative cell line BHK-21 which is fully permissive for these viruses. The viruses could penetrate normally into 3Y1 cells, but the subsequent viral genome and mRNA synthesis appeared to be strongly restricted and hence the viral protein synthesis remained at a very low level. The viral proteins, however, could assemble into subviral components such as nucleocapsids in a similar way to that in the permissive BHK cells. These data suggest that there may be some cellular factor(s) which regulate the transcription of the negative strand RNA viruses.

OP30.3

INVOLVEMENT OF CELL STRUCTURES AND FUNCTIONS IN RABIES INFECTION C. Conti*, F. Superti*, M. Divizia*, A. Panà*, H. Tsiang+, M. Orsi*
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The involvement of several cell structures and functions in rabies virus infection of CER cells has been studied by means of specific drugs capable of acting on membrane receptors (dithiothreitol, trifluoperazine), pH of intracellular vesicles (methylamine, monensin), cytoskeletal elements (vinblastine, colcemid, colchicine) and cell metabolism (sodium azide, 2-deoxyglucose). The viral infection was synchronized by a temperature shift: attachment was performed at 0°C and penetration at 37°C. The substances were present in the culture medium either before or during the attachment step or for the entire growth cycle. In this last condition all compounds tested, except microtubules disrupting agents, blocked the infection. Among the active compounds, only dithiothreitol was found to inhibit viral attachment. Drugs interfering with the endocytic pathway acted in the first hour after the temperature shift, whereas metabolic inhibitors were effective on later stages. Results here presented confirm that rabies virus enters CER cells by endocytosis and suggest that early stages of infection do not depend on cell energy or microtubule elements.

OP30.4

VESICULAR STOMATITIS VIRUS LEADER AND mRNA SYNTHESIS DIFFER IN THEIR ATP REQUIREMENT

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We reported previously that the pol R VSV mutants, as compared to the wild type (wt) virus, transcribe through the leader-N gene junction at much greater frequency in vitro and are less dependent on the beta-gamma bond of ATP for initiation of RNA synthesis. These phenotypes were mapped to a mutation(s) in the N protein. We describe here important differences in the kinetics of ATP utilization for plus and minus strand leader RNAs, synthesized by standard and defective interfering particles respectively, vs total RNA synthesized by standard wt and pol R virus. Both leader RNAs and total RNA from standard pol R approximate normal Michaelis-Menten kinetics whereas total RNA from standard wt VSV displays apparent positive cooperativity. We conclude that the progression of the VSV polymerase beyond the leader RNA on the genome template is dependent on a change in ATP utilization which is altered in the pol R mutants. These results suggest that ATP may interact with a regulatory site and modulate a switch from transcription to replication.

OP30.5

RELATIONSHIP BETWEEN RABIES VIRUS ATTACHMENT TO DIFFERENT CELLS AND THEIR SUSCEPTIBILITY TO INFECTION

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A possible relationship between plasma membrane receptor organization or other unrelated cellular factors and cell susceptibility to rabies virus was investigated in four different cell lines infected with rabies virus. HeLa and CER cells were fully susceptible to infection by CVS as judged by immunofluorescent assay, whereas a lower presence of immunofluorescent antigens was detected in EPC and A. albopictus cells. Competition experiments were performed in which the specific binding of rabies virus to CER cells was differently inhibited by membranes extracted from the various cells and by their protein and lipid components. Other experiments demonstrated that virus specific attachment was similar in all cell lines examined and the correlation between binding and the relative susceptibility to rabies infection of cells is discussed.

OP30.6

STUDIES ON THE INHIBITION OF PROCESSING OF U1 AND U2 RNA BY VESICULAR STOMATITIS VIRUS. Donna M. Eisenmann and Jack D. Keene. Department of Microbiology and Immunology, Duke University Medical Center, Durham, USA.

Upon infection of baby hamster kidney cells with Vesicular Stomatitis Virus (VSV), host macromolecular (RNA and protein) synthesis is shut down. We have recently shown that the earliest detectable effect of VSV on RNA metabolism involves a block in the maturation of U1 and U2 RNAs. RNA species of 3 and 10 additional 3' terminal nucleotides accumulated as precursor transcripts of U1 and U2 RNA, respectively. UV inactivation studies were used characterize the component of VSV responsible for this inhibition RNA of processing. VSV exposed to high doses of UV at 254nm (>70,000 ergs/mm^2) lost the ability to inhibit the maturation of U1 and U2 RNAs. The kinetics of the reversal of inhibition of U1 and U2 RNA processing did not appear to be the same as that for the reversal of inhibition of host transcription by UV light. We have developed new approaches for direct quantitation of intracellular leader RNA and will discuss correlations between leader RNA levels and the shut off phenomena.

OP30.7

REPLICATION AND PURIFICATION OF RABIES VIRUS IN McCOY CELL CULTURE. Y.L.Nogueira and J.M.M.Souza Felippe.Serviço de Virologia - Instituto Adolfo Lutz - São Paulo - BRASIL

Rabies virus propagation in various cell culture systems has been described,with different patterns of cytopathic effect.Tentatives of "in vitro" virus culture aim further standartization and development of vaccines for human beings and immunodiagnosis as well.

These experiments show that rabies virus presents a constant and characteristc cytopathic effects when inoculated in a culture of McCOY continuous cell line besides a high titer(DL50 10^8/0.03 ml in mice).Rabies virus grown in these cell cultures was purified in Sephadex G-75 and G-50 columns and banded in saccharose continuous density / gradient(10-60%) at 65000xg and its purity was / checked by immunoelectrophoresis and SDS-PAGE.

We tried to show objectively that these McCOY cells can be used to growing rabies virus and for further vaccine production as for rapid diagnosis of virus infection.

OP30.8

RABIESVIRUS GLYCOPROTEIN: EVIDENCE FOR TWO NOVEL ANTIGENIC SITES, AND SUBDIVISION OF SITE I ON THE BASIS OF COMPETITION ELISA AND ANTI-IDIOTOPE INHIBITION STUDIES.
H.Bunschoten, F.G.C.M.UytdeHaag, G.Droat, A.D.M.E.Osterhaus. National Institute of Public Health and Environmental Hygiene, Bilthoven, The Netherlands.

Using a panel of virus neutralizing mouse monoclonal antibodies raised against the PM strain of rabiesvirus, it was shown in a competition ELISA system that in addition to the five antigenic sites described by Lafon et al. (1), two additional non-overlapping sites may be present on the glycoprotein of rabiesvirus. These sites were present on various geographically different isolates and vaccine strains. In the same ELISA system it was shown that site I could be subdivided into two different probably partially overlapping sites. This was confirmed in inhibition studies with a panel of anti-idiotope monoclonal antibodies against site I-specific paratopes.

(1) Lafon et al. 1983, Dev.biol.Stand. 57:219.

OP31.1

VIRUS-SPECIFIC PROTEIN SYNTHESIS IN INSECT CELLS INFECTED WITH Choristoneura fumiferana NPV (CfMNPV). P. Jamieson, B.M. Arif, S.S. Sohi. Forest Pest Management Institute, Sault Ste. Marie, Ontario, Canada.

Virus-specific protein synthesis in infected cells was found to be temporally regulated during a 50 hour observation period. Infected cells were pulse-labeled at 2 hour intervals with ^{35}S-methionine followed by analysis on polyacrylamide gels. Approximately 35 virus-specific polypeptides ranging in molecular weight from 8K to 120K were found. Inhibition of cellular protein synthesis was seen at approximately 24 hours post infection which is later than that reported in T. ni or S. frugiperda cells infected with AcMNPV. The 10K protein, detected with ^{14}C-leucine, was not entirely devoid of methionine because it was labeled with this amino acid, though weakly. Pulse-chase experiments revealed post-translational cleavage of some polypeptides as well as modification of others by methylation, glycosylation etc. Virions labeled with mannose revealed that several structural polypeptides were glycosylated. The comparison of the occluded and non-occluded forms of the virus showed numerous quantitative and qualitative differences.

OP31.2

KASHMIR VIRUS MULTIPLICATION IN PUPAE OF THE HONEY BEE, *APIS MELLIFERA*.

D. Dall. Department of Plant Pathology, University of Adelaide, Waite Agricultural Research Institute, Glen Osmond, South Australia, 5064.

Kashmir bee virus (KBV) is widely distributed in honey bees in Australia, causing both overt and inapparent infections. Laboratory inoculations have shown that the ID_{100} of KBV is about 1×10^{-5} ng/pupa, and that viral multiplication is completed within 24 h post-inoculation. 250-450µg of KBV can be recovered from each infected pupa.

Microscopy has shown that virus replication occurs within cytoplasmic membranous vesicles in cells of the fore- and hind-gut, epidermis and tracheal epithelium, alimentary canal musculature, and in haemocytes, oenocytes and tracheal end cells. Infection is invariably associated with major cytopathological changes to host cells.

Glial elements of the nervous system show a consistent and severe pattern of degenerative change in KBV-infected pupae, even though thorough examination by EM has failed to demonstrate the presence of KBV in these cells. Such changes may be a cellular response to abnormal metabolic conditions induced by viral infection of the host.

OP31.3

AUTONOMOUS REPLICATING SEQUENCES (ARSs) OF CfMNPV, A BACULOVIRUS of CHORISTONEURA FUMIFERANA. H. Lee and P. Krell. Department of Microbiology, University of Guelph, Guelph, Canada.

A random Sau3A genomic library of CfMNPV DNA in the yeast ARS-selecting vector YIP5 (a pBR322 derivative coding for ura+) was constructed and used to identify ARSs in CfMNPV DNA by the ability of ARS+ recombinants to transform yeast strain YNN27 (ura3-52) selectable on ura- medium. Southern blot hybridization (SBH)of DNA from some of 152 stable ura+ yeast transformants probed with pBR322 DNA revealed that recombinant YIP5 DNA was in superhelical form and could transform E. coli HB101. The recombinant plasmids must thus have replicated autonomously and therefore contain ARSs. Two recombinant plasmids, pYCfSu 1 and pYCfSu 2, recovered from HB101 in this way contained CfMNPV DNA inserts of 8.0 and 8.5 Kb respectively, as judged by SBH. ARS activity in both recombinant plasmids was confirmed by their ability to transform yeast at a high frequency (350 ura+ colonies/µg DNA) as compared to YRP12 with a yeast ARS 1 (3000 ura+ colonies/µg DNA).

ARS activity was similarly detected in a 1.7 Kb Sau3A HzSNPV DNA fragment from the baculovirus HzSNPV from Heliothis zea.

OP31.4

MAPPING THE GENE ENCODING THE BASIC PROTEIN OF AUTOGRAPHA CALIFORNICA NPV. M.S. Hill-Perkins and R.D. Possee. NERC Institute of Virology, Mansfield Road, Oxford OX1 3SR UK.

Analysis of the mRNA produced throughout the infection cycle of the Autographa californica nuclear polyhedrosis virus (AcNPV) in Spodoptera frugiperda cells identified an abundant low molecular weight transcript. This message was mapped on the AcNPV genome using cloned virus DNA probes, representing the whole genome and Northern blotting. The region encompassing the source of the transcript was sequenced and found to consist largely of codons for the basic amino acid arginine. The 5' and 3' ends of the message were mapped using S1 nuclease analysis to accurately determine the size of the mRNA.

The precise temporal control of the mRNA was investigated and its susceptibility to metabolic inhibitors in order to define it as an early or late gene product.

The data obtained is consistent with this gene being responsible for the production of the major structural basic protein which binds to DNA in the virus nucleocapsid.

OP31.5

MOLECULAR STUDIES OF AN INSECT PICORNAVIRUS, CRICKET PARALYSIS VIRUS. L.A. King and N.F. Moore*. Department of Biology, Oxford Polytechnic, Oxford and *NERC Institute of Virology, Oxford, U.K.

Molecular studies were performed on a well characterised insect picornavirus, Cricket paralysis virus (CrPV). A complementary (c)DNA of the ssRNA genome was cloned into pBR322 by a hybrid RNA-cDNA cloning strategy. Overlapping clones spanning the estimated 8.5 kilo base-pair genome were obtained. Comparison of the restriction enzyme map with those of mammalian picornaviruses revealed no conserved cleavage sites.

Much of the cloned CrPV cDNA has been sequenced and this has allowed detailed comparisons to be made between the CrPV sequence and those of other mammalian picornaviruses. The results suggest that the insect picornaviruses and their mammalian counterparts are only distantly related, but have retained a similar genome organization and replication strategy.

CrPV-cDNA transfections into insect tissue culture cells were performed to examine the ability to replicate and form virus particles. This study was also extended to include mammalian tissue culture cells, to determine whether, in by-passing normal cellular receptors, an insect picornavirus can replicate in mammalian cells.

OP31.6

STUDIES ON SOME FACTORS AFFECTING THE REPLICATION OF EUXOA SCANDENS CYTOPLASMIC POLYHEDROSIS VIRUS (EsCPV) IN INVERTEBRATE CELL CULTURES. H. Rocheleau, M. Arella, S. Belloncik, Centre de recherche en virologie, Institut Armand-Frappier, Univ. du Québec, Laval, Canada.

Various factors affecting the replication of EsCPV in different invertebrate cell cultures monolayers and suspension cells were studied by microscopy, ELISA, dsRNA extraction and molecular hybridization. Besides others, cell strains from different tissue origin, initial cell concentration in suspension cultures prior to virus infection and concentration of cells used for seeding in monolayer cultures, temperature treatments of the infecting viruses suspensions and multiplicity of infection were considered both for the yield of non occluded viruses and polyhedra. Cloning was also performed from a mixte population of Heliothis armigera cell line in order to select elongated from round cells, the latter allowing a significant increase in polyhedra formation.

Since serial passages of EsCPV and other CPV is difficult for the lack of release of free viruses in the supernatant, we have established extraction procedures based on sonication, freeze-thawing and EDTA-trypsin treatments. All these treatments had little effect on EsCPV inoculum stability when assayed in a highly permissive Lymantria dispar cell line; however, the most efficient recovery of infectivity from cells was obtained by EDTA-trypsin treatment.

OP31.7

IDENTIFICATION AND CHARACTERIZATION OF AN EARLY GENE FROM THE BACULOVIRUS Autographa californica NUCLEAR POLYHEDROSIS VIRUS. B. Yu, A. Lu, E.B. Carstens. Department of Microbiology and Immunology, Queen's University, Kingston, Ontario, Canada.

Studies on a ts mutant defective in DNA replication and late protein synthesis has defined a gene regulating replication of Autographa californica nuclear polyhedrosis virus. Overlapping wt fragments, cloned into M13 vectors, and derived from sequential exonuclease digestion from both ends of a DNA fragment from 60.1 to 62.0 map units were used to fine map the ts lesion to a 300 bp site between 60.9 and 61.1 m.u.. A 4.6 kb early transcript has been physically mapped to the genomic region of the ts lesion. This region of wt AcMNPV has been sequenced and a single large open reading frame has been identified. The direction of transcription of the mRNA carrying this open reading frame, as well as the physical location of the 5' end, was determined by Northern blot analysis and primer extension reactions. A synthetic peptide was synthesized from the putative carboxy-terminal 15 amino acids of the open reading frame. Peptide-specific antiserum was generated in rabbits and its reactivity towards the immunogen was confirmed by ELISA. The peptide specific antisera was used in Western blots and immunoprecipitation assays to identify the presumptive early gene product. Supported by NRC.

OP31.8

RESTRICTION ENZYME PATTERN ANALYSIS OF THE DNAs OF THREE NUCLEAR POLYHEDROSIS VIRUSES. Chen Dihua, Zhao Huaiyu, Zhang Liren Wuhan Institute of Virology, Academia Sinica, Wuhan, P.R.China

The DNAs of three NPV genomes which extracted respectively from Boarmia obliqua W. NPV, Euproctis pseudoconspersa St. NPV and Prodenia litura (P.) NPV, have been studied by restriction enzyme analysis. The results showed that the restriction enzyme patterns of NPV-DNA among the three NPV infecting different host species were different from one another, but those between the two isolates of Boarmia obliqua were almost the same.

OP31.9

STUDIES ON DETECTION AND APPLICATIONS OF VIRAL PESTICIDES. M. Arella, and S. Belloncik. Centre de recherche en virologie, Institut Armand-Frappier, Laval, Québec, Canada.

The replication of potential viral pesticides, especially nuclear and cytoplasmic polyhedrosis viruses and granuloses, was studied for a number of years using traditional techniques of light and electron microscopy, cytochemical staining and serology.

In recent years a refined molecular approach was introduced by the widespread use of nucleic acids extraction, Restriction Enzyme Analysis (REA) and recombinant DNA technology.

The use of these techniques allowed us to compare geographical isolates of Pieris rapae granulosis virus (PrGV) and show minor modification by REA. Cloning of EcoR I restriction fragments in plasmid vectors was also used to prepare biotinylated DNA probes to follow GV infection in larvae from fields experimentally sprayed with granules. Molecular hybridization results showed that, in the original field of isolation, PrGV was persistently present during 3 years in the larvae, and the high efficiency of the experimental viral treatment for the biocontrol of the insect.

A study of Euxoa scandens cytoplasmic polyhedrosis virus (CPV) replication was also followed by cDNA probes. These probes labelled by nick-translation experiments either with ^{32}P-dNTP or biotinylated nucleotides were used to detect infection of CPV in larvae, cell cultures and for biosafety studies in non target organisms.

OP31.10

CELL-FREE PROCESSING OF BLACK BEETLE VIRUS COAT PROTEIN. D. Blackhurst, D. Hendry. Department of Microbiology, Rhodes University, Grahamstown, South Africa 6140.

Black beetle virus (BBV), a member of the Nodaviridae, is an insect-pathogenic small RNA virus with a bipartite genome. The smaller of the two genomic RNAs (RNA 2) codes for the 47K BBV coat protein precursor α, which during in vivo maturation is cleaved to produce the 43K coat protein β. BBV (strain W17) RNA 2 was translated in rabbit reticulocyte lysates. α was synthesised initially, but after 30 min a second protein ("β") emerged, while the level of α declined. "β" co-electrophoresed with authentic β and was immunoprecipitated by anti-BBV sera. The formation of "β" could be prevented by the addition of RNase (but not cycloheximide) to the lysate after 30 min, or by cooling the lysate to 1^{0}C. Sedimentation analysis indicated that the formation of "β" did not require its assembly into a larger viral substructure, nor did "β" appear to remain associated with the RNA. Serial dilution of lysate containing α resulted in the level of "β" declining with increasing dilution, indicating that the reaction was not autocatalytic.

These results suggest that BBV coat protein precursor α synthesised in rabbit reticulocyte lysate is cleaved to generate coat protein β. This cleavage requires the presence of intact BBV RNA 2, but requires neither continued translation nor assembly of β into a viral substructure.

OP31.11

GENOMIC MAPPING OF VIRAL (POLYDNAVIRIDAE) TRANSCRIPTS CORRELATED WITH ESCAPE OF PARASITIC WASPS FROM HOST INSECT IMMUNE RESPONSE. D. Jones, Department of Entomology, University of Kentucky, Lexington, KY, USA.

A polydnavirus which replicates within the ovaries of a parasitic wasp (Chelonus sp.) is injected into host (Lepidoptera) eggs during oviposition (1). The DNA of the segmented genome of the virus contains at least 17 different circular, double-stranded size classes, most of which is recovered in relaxed form following purification on CsCl gradients (2). Stung hosts insects exhibit developmental redirection by precocious initiation of metamorphosis during the penultimate stadium, even when the internal parasite is dead and encapsulated by host hemocytes. Comparative Northern dot blot analysis of mRNA from hosts in which the parasite has survived or died showed the presence of mRNA complimentary to virus DNA only in those hosts in which the parasite has survived (Fig. 1). The genomic organization of the virus has been studied to facilitate mapping of the transcripts on the virus. Some sequences appear to share homology with sequences on other other genomic segments. Fig. 1. Blot of mRNA d) normal larvae; stung a) permissive, c) nonpermissive hosts; b) viral DNA. Probe-total viral DNA

1) Jones et al. 1986, J. Invert. Path. 47:105. 2) Jones and Zador 1986, J. Cell. Biochem., supp. 10C: 88. (NIH GM33995).

OP32.1

ANTIGENIC AND GENETIC CONSERVATION OF H3 INFLUENZA VIRUS IN WILD DUCKS. H. Kida[1], Y. Kawaoka, C.W. Naeve, and R.G. Webster. St. Jude Children's Research Hospital, Memphis, TN, USA, [1]Faculty of Veterinary Medicine, Hokkaido University, Sapporo, Japan.

The hemagglutinins of H3 influenza viruses isolated from migratory ducks during the period 1977 to 1985 were analyzed antigenically and genetically. Antigenic analysis using monoclonal antibodies showed that antigenic drift occurred extensively in human strains, whereas the hemagglutinins of duck viruses were highly conserved and that the hemagglutinins of duck viruses were closely related to that of human 1968 H3 prototype strains. Nucleotide sequence analysis of seven duck H3 hemagglutinin genes showed that the nucleotide variation rates among 6 duck isolates and between these duck isolates and Aichi/68 virus were extremely low (0.17-0.34% per year). The deduced amino acid sequence revealed amino acid changes randomly distributed throughout the molecule and not confined to antigenic sites. These findings indicate that the duck virus hemagglutinin genes are maintained with very low variation rates without antibody selection pressure and that viruses of different lineages cocirculate.

OP32.2

INFLUENZA ECOLOGY IN ITALY

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The widespread distribution of influenza A viruses in lower mammals and birds and the possibility that these viruses may be involved in the origin of new human pandemic strains have led to increased efforts to understand the natural history of influenza viruses in animals. In Italy there had been little indication of animal influenza until 1977. Since then,a continue virological and serological survey of influenza circulation in non-human hosts has provided incidence of clinical and sub-clinical circulation of influenza A(HINI-H3N2) viruses in lower mammals and birds. Detailed antigenic analysis and preliminary biochemical comparison of a number of viruses isolated from pigs, in Northern and Central Italy, were undertaken in attempts to establish the genetic relationship of viruses in one species to those appearing in others.

OP32.3

ENHANCED DETECTION OF VIRUSES IN WASTEWATER BY AN IMMUNOPEROXIDASE ASSAY ON MA-104 CELLS. P.Payment and M.Trudel. Virology Research Center, Institut Armand-Frappier, Laval, Quebec, Canada H7N 4Z3

Because many viruses do not induce any cytopathic changes when they infect cells, most assays for the detection of viruses in water underestimate the viral content of the waters tested. An immunoperoxidase method based on the use of human immune globulins as the source of antibodies and two blind passages on MA-104 cells and developed by the authors has been used to evaluate the viral content of wastewaters. This method has been proven up to 6 times more sensitive than a cytopathic effect assay on MA-104 cells and up to 50 times more sensitive than a cytopathic effect assay on BGM cells. The viral content of waste waters measured with this new method was shown to be up to 50 times higher than what was reported earlier. The average viral content of weekly samples of raw sewage was 900 mpniu/L while treated wastewater samples contained an average of 106 mpniu/L. Evaluation by cytopathic effect on BGM cells would have resulted in the detection of only 85.5 and 2.0 mpniu/L respectively, a striking underestimation of the viral content of these treated waters. The viruses detected are probably non-cytopathogenic viruses such as reoviruses and rotaviruses or slow growers such as hepatitis A virus and adenoviruses.

OP32.4

EXPERIMENTAL STUDIES ON THE SURVIVAL OF ENTERIC VIRUSES IN WATER.

K. MATSUURA, O. MORITA & H. UETAKE. Toyama Institute of Health, Nakataikoyama, Kosugi-machi, Toyama 939-03, JAPAN.

Since the river waters in Toyama Prefecture have been found to be contaminated with enteric viruses such as picornaviruses and reoviruses, we have attempted to determine the survival time of the viruses in the river water in order to clarify ecology of these viruses in the environment.

Each of poliovirus type 1 (Mahoney strain), reovirus types 1, 2 and 3 (Lang and Any strains, our isolate K-160 and Dearing strains) was inoculated into the river water and stirred, respectively. Aliquots were distributed into bottles, that were shielded from light with aluminum foil, and kept at 5°C, 15°C or 23°C for 23 months, during which time virus titers were determined at appropriate time intervals.

At 5°C most of the virus strains were found to survive for 23 months, though their final titers varied with the virus strains employed and the quality of the river water. At 15°C all the strains were found to survive for 10 months, after which time many were inactivated gradually except reovirus types 1 and 2. At 23°C they lost their infectivity slowly and gradually in 18 months.

As for the survival time of viruses in water, effects of many factors such as water quality, water temperature, light, the presence or absence of other micro-organisms, etc. have to be taken into considerations, but the present experiments indicate that poliovirus and reoviruses are capable of surviving for a very long time in the river water.

OP32.5

TRANSOVARIAL TRANSMISSION IN SAND FLIES (DIPTERA, PSYCHODIDAE) EXPERIMENTALLY INFECTED WITH PHLEBOVIRUSES. M.G. Ciufolini, M. Maroli, E. Guandalini and P. Verani
Laboratories of Virology and Parasitology, Istituto Superiore di Sanità, Rome, Italy.

In order to demonstrate the possibility of a transovarial transmission cycle, laboratory reared Phlebotomus perniciosus were infected either by intrathoracic inoculation or by membrane feeding with two Phleboviruses (Toscana and Arbia), isolated in Italy from this sand fly species. Both viruses were found to be transovarially transmitted respectively to two and three consecutive adult generations of parenterally infected sand flies. No evidence of transovarial transmission was instead demonstrated when an innatural vector (e.g. P. papatasi) was inoculated with both viruses. Moreover no infection signs were observed in F_1 adults resulting from the first gonotropic cycle of females infected by the oral route. These results confirm the hypotesis that transovarial transmission could be one of the mechanisms by which Phleboviruses are maintained in nature.

OP32.6

MOLECULAR MECHANISM OF RESISTANCE OF PLANT VIRUSES TO ULTRAVIOLET RADIATION. A.G.Trubitsin
Institute of Biology and Pedology, Far-eastern Science Centry of the Academy of Sciences of the USSR, Vladivostok, USSR.

Plant viruses lack the photoreparation mechanism, but they has capacity to resist UV radiation. Photodamages of nucleic acids are due to some photochemical reactions: dimirisation of pyrimidine bases, covalent bounding of polynucleic chains, hydration of pyrimidine rings and formation of pyrimidine adducts. Specific conditions are needed to these reactions. However, the coat proteins of simplest viruses press on the RNA chain such the secondary structure, that proper conditions are absent. As a consequence, 3 most harmful photochemical reactions don't take place in virion RNA. Only pyrimidine adducts form without any restrictions. The coat protein has its own protection mechanism. The UV radiation energy absorbed by proteins disactivate in 3 mutually competitive processes: erradiation (fluorescence), thermodissipation and photochemistry. Coat proteins has an extremely high ratio of rate either of erradiation (TMV, PVX and others), or thermodissipation (CCMMV, BMV and others) processes. As a result, photochemical reactions lacked an activation energy and so decrease.

OP32.7

MOSQUITO-VIRUS INTERACTIONS IN THE TRANSMISSION OF EASTERN EQUINE ENCEPHALOMYELITIS VIRUS. T. W. Scott, L. H. Lorenz, S. C. Weaver, W. S. Romoser, and K. Lerdthusnee. Dept. of Entomology, University of Maryland, College Park, Maryland, USA, and Dept. of Zoology, Ohio University, Athens, Ohio, USA.

Recent studies on eastern equine encephalomyelitis (EEE) virus in its enzootic mosquito vector, Culiseta melanura, show that this virus can be transmitted more quickly than was previously thought and that virus replication in midgut epithelial cells is associated with pathological changes. Examination of dissected organs for infecticus virus or virus antigen shows that EEE virus infects mosquito salivary glands after 2-3 days of extrinsic incubation (EI). Transmission electron microscopy (TEM) studies show that virus is released into the salivary matrix in 48 hr. At 25°C virus can be transmitted after 48-72 hr of EI. Rapid mosquito infection could increase the probability of transmission and the potential for rapid outbreaks of disease.

Examination of posterior midgut epithelial cells by TEM reveals virus-associated changes during the 2nd-7th day of EI. In infected cells, intercellular spaces and the basal labyrinth are swollen and contain copious amounts of virus. Dark staining of infected cells implies an increase in cytoplasmic density. Changes are associated only with high to moderate titered blood meals. In some specimens, intercellular spaces extend through the zonula continua and brush border. Occasionally, cells containing virus can be seen in the gut lumen and appear to be morbid.

OP32.8

DISTRIBUTION OF COLIPHAGES AND HUMAN ENTERIC VIRUSES IN THE PARTICULATE PHASE OF RIVER WATER. P.Payment, E.Morin and M.Trudel, Virology Research Center, Institut Armand-Frappier, Laval, Quebec, Canada H7N 4Z3.

Viruses in surface waters are colloidal particles that interact with all other particulate material in the water. Because these particulates are important during treatment of drinking water and wastewater treatment, we have studied the distribution of viruses in the particulate phase of river water. Large volume of river water (300 to 400 liters) were filtered through sequential filters of decreasing porosity and treated with Tween-80 to eliminate virus adsorption. The porosity of these cartridge filters (Filterite) was 100, 30, 10, 3, 1, 0.45 and 0.25 micron. The filtrate was then conditioned to pH 6 and 0.05M magnesium chloride and filtered on a 0.25 micron Duo-Fine filter to adsorb free viruses. Human enteric viruses were detected by immunoperoxidase on MA-104 cells while coliphages were detected on ATCC 13706 E.coli C host cells. Only 27% of the coliphages were associated with particulates 3 microns while 64% were free. Human enteric viruses were found in similar distribution pattern: 60% were free while 27% were found associated with particulates less than 3 microns. Most of the viruses, bacteriophages or human enteric viruses present in surface waters appear to be free or associated with particulates less than 0.25 micron in diameter. This observation is important because in water treatment small particulates are the most difficult to eliminate.

OP32.9

DETECTION OF NON-HUMAN ENTERIC VIRUSES IN THE WATER OF A RIVER SYSTEM. P.Payment, F.Affoyon, M.Trudel and E.DiFranco. Virology Research Center, Institut Armand-Frappier, Laval, Quebec, Canada H7N 4Z3.

Virological analysis of surface and groundwater samples in a highly agricultural area was undertaken to evaluate the impact of pig raising farms on water quality. The l'Assomption river and its affluents, situated near Montreal (Prov.Quebec, Canada) provided an excellent site for these studies. Twenty-liters samples of river water and 200-liter of underground water were collected weekly for two years and concentrated by the Viradel method on electronegative filters. Concentrates were analyzed on porcine kidney cells (PK-15) and porcine thyroid cells. Viruses were detected by ELISA (reoviruses), cytopathic effect, immune electron microscopy and immunoperoxidase. According to the sampling site, from 33 to 81% of the samples were positive for animal viruses (i.e. reoviruses or enteroviruses) at concentrations varying from 0.2 to 200 mpniu/L. At the mouth of the river before its discharge in the St-Lawrence river, the average reovirus concentration in the water was 5.88 mpniu/L and for enterovirus was 0.93 mpniu/L. Two out of 22 samples of groundwater were found to contain porcine enteroviruses at 11 and 5 mpniu/100L. The presence of such high virus numbers in both surface and groundwater constitutes a high risk for animal herds in this area as these animals are usually given groundwater or treated surface water as drinking water.

OP33.1

BORDER DISEASE VIRUS OF SHEEP IS A VARIANT OF BOVINE VIRAL DIARRHEA VIRUS

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Border disease (BD) is a congenital viral infection of sheep and goats caused by a non-arbo virus of the Togaviridae family. This virus has long been known as a separate entity that cross reacts antigenically with hog cholera virus of swine and bovine viral diarrhea (BVD) virus of cattle. We examined two different isolates of BD virus for comparison with BVD virus to identify the cross reacting antigens between these viruses. Radioimmunoprecipitation of viral polypeptides using BVD and BD specific antisera revealed the cross reacting viral polypeptides of both viruses from infected cells. Our results indicate that BVD and BD viruses are similar in their polypeptides with the exception of the viral glycoprotein. Of three different BVD strains (NADL, Singer, NY1) compared with BD virus, the non-cytopathic BVD strain NY1 seemed to be more closely related to BD virus. In view of our recent data, we propose that BD virus is not a separate viral species but rather is a variant of BVD virus.

OP33.2

DNA SEQUENCE RELATEDNESS OF THE LENTIVIRUS VISNA AND HUMAN IMMUNODEFICIENCY VIRUS (HIV) IS MINIMAL.

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Computer analysis of the sequence relationships between the lentiviruses visna and HIV have been rigorously performed. While taxonomically related, DNA sequence homology is limited primarily to the pol gene, in contrast to published reports from other investigators based on low stringency hybridizations of the viral DNAs maintained in phage lambda. Analyses have been performed by Wilbur-Lipman alignment, by Needleman-Wunsch alignment, and by homology matrix analysis of DNA sequences, with similar negative results. When amino acid sequences implied by the orfs were analyzed under the same conditions, as well as with amino acid chemical equivalences, significant matches were formed primarily in the polymerase region, as well as the Q and S orfs.

OP33.3

SEQUENCE ANALYSIS OF THE HUMAN CYTOMEGALOVIRUS GENOME AND COMPARISON WITH OTHER HERPES VIRUSES.

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Human cytomegalovirus (HCMV) is a beta-herpesvirus and has a genome size of approximately 235Kb. Sequence analysis of the AD169 strain has revealed distant homology with the genomes of the alpha-herpesviruses VZV and HSV and the gamma-herpesvirus EBV. Several homologous blocks of sequence have been detected by a comparison of the amino acid sequences of open reading frames deduced from the DNA sequence. Other regions, e.g. the 35 Kb short unique sequence, show no homology either at the amino acid level, or in the overall organisation of reading frames. The homologous blocks of sequence are not colinear with other herpes virus genomes indicating that a complex series of genetic rearrangements have occured in the evolution of herpesvirus genomes. A detailed analysis of the open reading frames and their organisation will be presented.

OP33.4

RESTRICTION ENDONUCLEASE ANALYSIS OF DENGUE 2 ISOLATES.

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Dengue viruses have been divided into genetic races (topotypes) by oligonucleotide fingerprint analysis. Topotype analysis is useful in understanding the distribution and flow of these viruses around the world. We have evaluated the usefulness of restriction enzyme (Hae III) cleavage of complementary DNA as an alternative to the cumbersome and time-consuming oligonucleotide fingerprinting procedure.

Comparison by polyacrylamide gel electrophoresis of Hae III fragments of complementary DNA among five Dengue 2 topotypes revealed a general correlation with oligonucleotide fingerprint homologies. The numbers of shared Hae III fragments within topotypes ranged from 70% to 100%, while homologies among different topotypes ranged from 10% to 50%. Homologies with other dengue serotypes were less than 10%. By oligonucleotide fingerprint analysis, no homologies can be seen among different dengue typotypes. Therefore, Hae III analysis differentiates the same topotypes as oligonucleotide fingerprinting, while at the same time showing lower but significant homologies among topotypes. The broader homology among topotypes shown by Hae III analysis may be useful in determining evolutionary relationships among different virus populations.

OP33.5

GENOME ORGANIZATION OF BARLEY YELLOW DWARF VIRUS.

W.A. Miller, P.M. Waterhouse, W.L. Gerlach. CSIRO Division of Plant Industry, Canberra, Australia.

Barley yellow dwarf virus (BYDV) is the most widespread virus of cereals crops worldwide. To begin molecular characterization of this virus, we have determined the complete nucleotide sequence of the PAV serotype of BYDV. Open reading frames are evident and some genes have been identified. The 5'-most gene is the putative RNA-dependent RNA polymerase, which shows striking sequence homology with carnation mottle virus polymerase. Other genes are expressed via subgenomic mRNAs. The coat protein (22 kd) of BYDV is located near the middle of the genome, unlike other RNA viruses which use subgenomic mRNAs. There is potential for translational readthrough of the coat protein gene stop codon to yield a 69 kd protein. An overlapping reading frame which could code for a 17 kd protein is contained within the coat protein gene. The coat protein shows structural similarity to that of southern bean mosaic virus. The possible role of viral gene products in aphid transmission will be discussed. The genome will be compared in evolutionary terms with partial sequences of other luteoviruses.

OP33.6

DULCAMARA YELLOW FLECK VIRUS /DYFV/, A NEW MEMBER OF THE TOBAMOVIRUS GROUP IN HUNGARY.

P.Salamon[1], L.Beczner[1], R.I.Hamilton[2], Plant Protection Institute, Hungarian Academy of Sciences, Budapest, Hungary[1], Agriculture Canada Research Station, Vancouver,Canada[2]

A mechanically transmitted rod-shaped plant virus of ca. 300 x 18 nm in size has been isolated from and found to be widely distributed in native and cultivated Solanum dulcamara in Hungary. The virus was characterized by host range, serological, electrophoretic, cytological and sedimentation studies in comparison with standard and Hungarian isolates of eight tobamoviruses / e.g. TMV, ToMV, PTMV, RMV, PMMV, ORSV, SHMV, CGMMV /. Based on the results the dulcamara tobamovirus could be identified as a new member of the tobamovirus group. Its unique taxonomical status was also confirmed by RNA-DNA hybridization tests. For the new plant virus, the name dulcamara yellow fleck virus is proposed. Spontaneous mutants of the DYFV were also separated from a tobamovirus complex isolated from pepper / Capsicum annuum / in Hungary. These mutants differ from the wild isolates and from each other in the trait to induce local lesions of various sizes and/or sytemic infection in tobacco species and cultivars carrying the N-gene for tobamovirus resistance.

OP33.7

A VIRUS INFECTION IN THE GREEN ALGA COELASTRUM MICROPORUM.
K.Y. Chan. Department of Biology, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong.

Electron microscopic study of synchronous cultures of the green alga Coelastrum microporum growing in liquid medium found that some cells contained clusters of virus-like particles in the cytoplasm. These particles were tail-less, polygonal in shape with a prominent capsid of about 90 nm in diameter. These viral particles have capsids with an outer electron-dense layer and contain differently stained materials from heavily stained to rather translucent thus probably representing different stages in their assembly. Clusters of viral particles were usually found only near the chloroplasts which located in the peripheral areas of the algal cell, and the virus was never found to associate with the nucleus of the algal cell. The cytoplasm and organelles of the infected cells showed considerable evidence of disruption and breakdown.

Although lysis of the algal cells was observed in young cultures, the relationship between cell lysis and the presence of the virus in the algal cell has not been established. This virus was found to be capable of infecting two other species of Coelastrum.

OP33.8

COAT PROTEIN SEQUENCE HOMOLOGY AS A CRITERION FOR CLASSIFICATION OF THE POTYVIRUS GROUP OF PLANT VIRUSES.
D.D. SHUKLA, D.R. Hewish, K.H. Gough and C.W. Ward, CSIRO, Division of Protein Chemistry, Parkville, 3052, Australia.

Potyvirus group is the largest and economically most important of the plant virus groups. Past attempts to evaluate criteria to differentiate individual potyviruses, to define strains and to divide potyviruses into subgroups have been unsatisfactory. Recent findings on the coat protein sequences and comparative binding of monoclonal antibodies to virus particles before and after removal of surface epitopes by protease digestion suggest that amino acid sequence data could provide a rational basis for the classification of potyviruses. Our data on ten strains of four distinct potyviruses together with that published for three others reveals that biologically and serologically distinct members exhibit about 60% homology with major differences in length and sequence at their N-termini. Related strains exhibit over 90% sequence homology and have N-terminal sequences that are almost identical. Proteolytic digestion of virus particles revealed that the N- and C-termini of the coat protein are exposed on the virion surface.

We propose that potyviruses with a unique N-terminus and with about 60% coat protein sequence homology should be regarded as species, those with a sequence homology of over 90% as strains and biologically distinct strains with identical coat protein sequences as pathotypes.

OP34.1
THE ASSOCIATION OF SINDBIS VIRUS-SPECIFIED PROTEINS WITH THE VERO CELL CYTOSKELETON.
Mah Lee NG and Coreen Ooi, Department of Microbiology, Faculty of Medicine, National University of Singapore, Lower Kent Ridge Road, Singapore 0511, Republic of Singapore
When the infected cell cytoplasm was extracted with Triton X-100, the core protein was seen by page to be associated with the remaining cell cytoskeleton. The highest intensity was observed at 12 hour P.I., the peak virus replication. Two cytoskeletal-disrupting drugs were used, namely, vinblastin (disrupt microtubulin) and cytochalasin B (affect microfilaments) in an attempt to determine which component of the cell cytoskeleton was associated with the core protein. The two drugs did not alter the association of the core to the cytoskeleton, thus the core protein was probably anchored onto the intermediate filaments. However, the 93k protein precursor was not cleaved into the PE2 and E1 proteins in the presence of cytochalasin B. In the vinblastin-treated infected cells, there was no accumulation of the 93k precursor protein. Currently, it appears that all three main components of the vero cell cytoskeleton are involved in the processing or accumulation of the sindbis virus-specified proteins.

OP34.2
MICROTUBULES IN THE REPLICATION OF KUNJIN VIRUS.
Saw See Hong and Mah Lee NG, Department of Microbiology, Faculty of Medicine, National University of Singapore, Lower Kent Ridge Road, Singapore 0511, Republic of Singapore.
Thin-sections of Kunjin virus-infected vero cells at 30 hours post-infection (P.I.) revealed several interesting virus-induced structures. There was induction of vesicles, aggregation of smooth membraneous structures and microtubular paracrystals.

The microtubular paracrystals were found to be induced by Kunjin virus from 15 hours P.I. and also frequently associated with virus particles and other virus induced structures. Vinblastine sulphate (VS) was also able to induce the formation of paracrystals in vero cells similar to those observed in Kunjin virus-infected cells. The intracellular and extracellular virus titres examined were found to be reduced by almost 50%.

Cytoskeletal preparation of 35 S-Methionine labelled Kunjin virus-infected vero cells followed by SDS-polyacrylamide gel electrophoresis showed several viral proteins associated with the host cytoskeleton. We postulate that certain components of the cytoskeleton, most significantly the microtubules is involved in the replication Kunjin virus.

OP34.3
EXPRESSION OF COAT PROTEIN GENES IN TRANSGENIC PLANTS CONFERS PROTECTION AGAINST AlMV, CMV AND PVX.
C. Hemenway, K. O'Connell, W. Kaniewski, R-X. Fang*, M. Cuozzo*, N-H. Chua*, and N. Tumer. Monsanto Company, St. Louis, MO,-The Rockefeller University, New York, NY.
As previously demonstrated with TMV, cross-protection can be achieved by genetically-engineering plants to express the viral coat protein gene. To determine whether this approach will apply to viruses from different groups, we have cloned the coat protein (CP) genes from AlMV, CMV, and PVX, and have introduced chimeric genes into plants. Progeny from transgenic plants and control plants were subsequently infected with the appropriate virus and evaluated for appearance of symptoms. Virus levels were quantitated by ELISA analysis. In plants expressing AlMV or CMV CP, symptom development on inoculated leaves was delayed and a large percentage of plants did not develop symptoms during the experiment. Most transgenic plants expressing PVX CP developed symptoms on inoculated leaves, but the numbers of lesions were fewer than on control plants. Further, in plants expressing CMV or PVX CP genes, systemic infection was delayed and some plants did not develop systemic lesions up to several weeks post-infection. These results indicate that genetically-engineered cross protection is a method that can be generally applied to protect plants against different viruses.

OP34.4
ON THE SDS-PAGE ANALYSIS OF 4 TYPES OF CELL CULTURE INFECTED WITH 4 STRAINS OF HERPES SIMPLEX VIRUS
Zhang Tian-ming, Hsiang Chin-min, Feng Jue-sun. Virus Research Institute, Hubei Medical College, Wuchang, Hubei, PRC
Four types of cell culture, namely, Hep-2, HeLa, human embryo lung fibroblast and chicken embryo fi-broblast were infected with 4 strains of HSV, i.e. CC21, F,W, and G, the formal 2 of type 1 and W, G of type 2. The SDS-PAGE maps showed difference between the maps form at 4h after infection and th-at at twenty four hours after virus infection, th-e electrophoresis maps showed a difference betwee-n infected and uninfected cells. In the chicken embryo fibroblast culture, there were difference between maps of HSV-1 and that of HSV-2. This was to mean that by the chicken embryo fibroblast system HSV-1 could be differentiated from HSV-2 by th-e SDS-PAGE method. Therefore the PAGE technique might be used as a method to classify the types of herpes simplex virus.
After sucrose gradient density centrifugation the SDS-PAGE technique showed 12 bands in HSV-1 and 15 bands in HSV-2 respectively. A discussion was made on some problems of selection of types of cel-l culture in case the vaccine production is consi-dere.

OP34.5
CONCENTRATION AND PURIFICATION OF LOCAL STRAINS OF HERPES SIMPLEX VIRUS. Zhang Tian-ming, Hsiang Chin-min, Feng Chio-sun. Virus Research Institute, Hubei Medical College, Wuchang, Hubei, PRC
This paper reports the results of a study on con-centration and purification of local strains of h-erpes simplex virus. The experimental data may be summerzed as follows: The PFU titer of the local strains of HSV-1 cc21 and HSV-2 W cultured in Hep-2 cell were raised from $4.5X10^6$/ml after concentra-t by PEG 20,000 dialysis. After a futher procedur-e to purify the PEG concentrated fluid sucrose gr-adient centrifugation, the efficiency of purifica-tion was evaluated by PFU-Counts, electron micro-scopic examination and ultraviolet absorption spe-ctrometry. In addition to the typical form of the herpesvirus virion, the HSV-2 W strain showed an unusual morphology structure with either 3 nucleo capsides in an envelope or 3 cutize virions in one membrance structure. Perhaps the morphogenesis of HSV may be not so simple as described in the lite-rature. This deserves a further study.

OP34.6
THE INFLUENZA C VIRUS GLYCOPROTEIN (HE) EXHIBITS RECEPTOR-BINDING (HEMAGGLUTININ) AND RECEPTOR-DESTROYING (ESTERASE) ACTIVITIES.
R.Vlasak, M.Krystal, M. Nacht and P. Palese. Mount Sinai School of Medicine, 10029 New York.
A cDNA copy of the influenza C/Cal/78 virus gene encoding the viral glycoprotein was expressed in CV-1 cells using an SV40 vector. Cells infected with the SV40 recombinant virus exhibited C virus specific receptor binding (hemagglutinin) and receptor destroying (esterase) activities. In analogy to the parainfluenza HN protein, the influenza C virus glycoprotein was termed HE, because it possesses hemagglutinin (H) and esterase (E) activity. Viral esterase activity was measured using as substrates bovine submaxillary mucin or a low molecular weight compound p-nitrophenylacetate. Several monoclonal antibodies directed against the viral HE protein hemagglutination inhibiting activity, but no esterase inhibition. On the other hand, phenylmethylsolfonylfluoride, a protease inhibitor, inhibited esterase activity, but not hemagglutination activity.

OP34.7

RABIES VIRUS SELECTIVELY IMPAIRS DISTINCT NEURONAL FUNCTIONS IN NEUROBLASTOMA CELLS OF DIFFERENT ORIGIN.

M.Halbach, I.Berners. Psychiatrische Klinik, University of Duesseldorf. D4000 Duesseldorf,FRG.

Persistent infection of murine neuroblastoma x glioma hybrid cells with rabies virus hep flury strain causes highly specific damage to typical neuronal membrane located functions, reflected by a reduced cellular capability to react to the binding of opiates to their receptors with characteristic changes in the enzymatic activity of receptor linked adenylatecyclase. The functions of receptors for acetylcholine (mAChR), catecholamines, prostaglandins remain unaltered.

The infection of the human neuroblastoma cell line IMR 32 with the same rabies virus however resulted in a complete loss of the mAChR function. Receptors for prostaglandins and catecholamines were completely unaffected.

The distinct but highly specific sites of viral attack in cells of comparable neuronal character but different origin demonstrate that the specificity of viral induced damage to membrane located function depends from subtle membrane properties probably different between cells of similar character rather than from the type of virus establishing persistent viral infection.

OP34.8

DETECTION OF INFECTIOUS PANCREATIC NECROSIS VIRUS (IPNV) IN INFECTED CHSE-CELLS BY MEANS OF NUCLEIC-ACID HYBRIDIZATION.

L.S.Havarstein, K.E.Christie and *C.Endresen. Norbio A/S and *Center of Biotechnology, University of Bergen, Norway.

IPNV is a ds-RNA virus which can cause infection in several species of fish, including Atlantic salmon (Salmo salar).In Norway the virus has been detected in several hatcheries, and there are proofs indicating that few Norwegian hatcheries are free of this virus. The consequences of all this for the fish farming industry have not been made clear as yet. In order to get a better view of the situation, it is important to develope reliable diagnostical methods.

We have used nucleic-acid hybridization (slot-blot) to demonstrate the presence of IPNV in infected CHSE-cell cultures. It was shown that one million tissue culture infectious doses could be assayed by this method when a synthetic oligonucleotide (99 bp) was used as a probe.

The large number of serologically different IPNVs found is a disturbing factor in the diagnostics based on immunological methods. The probe used in this study is homologous to a gene coding for an internal protein (VP 31) assumed to be conserved among the various strains of IPNV. With this probe we were able to detect both the major serotypes (I,II and III) and three isolates of IPNV from local farm stocks.

SATELLITE SYMPOSIUM

SYMPOSIUM SATELLITE

SS1.1

OPTIMIZATION OF LIPOSOMAL CARRIERS FOR ANTIVIRAL THERAPY. M. Kende, M. Contos, W. Rill and P.G. Canonico. U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland 21701-5011, U.S.A.

Liposome-mediated delivery of antiviral compounds was optimized for encapsulation procedures, encapsulation yield/stability, and homogeneous size distribution to improve antiviral efficacy.

With the Liposomat (Dianorm, Switzerland) continuous flow plate-dialyser unit, an 80% size uniformity of unilamellar vesicles was obtained with egg yolk lecithin and n-octylglucoside detergent (lipid:detergent ratio, 0:2). The average particle size was 180 nm measured by LASER beam submicron particle analyzer (Coulter Model N4). However, with this method the encapsulation yield of the antiviral ribavirin was 4-5-fold lower than that obtained by rotary evaporation (RE). Multilamellar vesicles (MLV), prepared using RE with dipalmitoyl phosphatidylcholine (DPPC)-cholesterol (CL)-dicetylphosphate (DP) in a molar ratio of 2.0:1.5:0.22 had a size distribution of 0.02-3μm. The antiviral efficacy of ribavirin encapsulated in RE-prepared MLV was substantially enhanced in comparison with the non-encapsulated drug against Rift Valley fever (RVF) or influenza A viral infections in mice. High encapsulation yield (30-50%) of a quinolinamine derivative was obtained in MLV using RE with DPPC-CL-DP or phosphatidylserine-CL. Comparative efficacy studies against lethal RVF virus will be described.

SS1.2

LOCALIZED DELIVERY OF ANTIVIRAL DRUGS TO TREAT HERPES VIRAL INFECTIONS. Deborah A. Eppstein, Y. Vivienne Marsh, Philip L. Felgner, Elizabeth B. Fraser-Smith, and Thomas R. Matthews. Syntex Research, Palo Alto, CA 94304, USA.

It has been difficult traditionally to develop nontoxic antiviral drugs, due to the similarity of biosynthetic mechanisms employed by viruses and their host cells. Although significant advances have been made in developing drugs to inhibit herpes viruses by exploiting selective drug activation by viral-specific enzymes [acyclovir, gancyclovir (DHPG)], room for improvement still exists for reducing the potential of systemic toxicities, especially for long term administration of nucleosides. Accordingly, we have begun to study localized delivery routes for treating viral infections of a circumscribed nature (eg, for genital or pulmonary viral infections, treat respectively via topical application, or aerosolize drug into the lung). We have also studied unique liposome preparations for enhancing topical penetration of interferon, for use in synergistic applications with acyclic guanine nucleosides to treat genital HSV-2 infections. Results from these studies will be presented.

SS1.3

DEVELOPMENT OF INJECTABLE, CONTROLLED-RELEASE POLY(I·C) MICROCAPSULES FOR THE INHIBITION OF VIRAL REPLICATION. T.R. Tice, R.M. Gilley, W.M. Shannon, K.L. Pledger, L.D. Swift. Microencapsulation Division, Southern Research Institute, Birmingham, Alabama, U.S.A; and M. Kende, U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland, U.S.A.

Polyriboinosinic-polyribocytidylic acid [poly(I·C)] is a potent inducer of interferon, which is a nonspecific inhibitor of viral replication. One of the major disadvantages of using poly(I·C) therapeutically, however, is its poor bioavailability because of its rapid clearance from the body. In an attempt to enhance the bioavailability of poly(I·C), an injectable, biodegradable microcapsule formulation has been developed which is designed to release poly(I·C) at a preprogrammed rate and duration. This microcapsule formulation consists of poly(I·C) encapsulated in poly(DL-lactide-co-glycolide), a biocompatible, biodegradable polyester. When tested in vitro, this formulation releases poly(I·C) at a controlled rate for one to two weeks. Mice treated with poly(I·C) microcapsules and then challenged with Rift Valley Fever virus showed significantly better survival (90%) over controls (10%) at 3 weeks post microcapsule treatment.

This work was supported by the U.S. Army Medical Research Acquisition Activity under Contract DAMD17-86-C-6044.

SS1.4

TREATMENT OF JAPANESE ENCEPHALITIS IN A MURINE MODEL WITH A RIBAVIRIN PRODRUG. M.A. Ussery, J.A. Secrist III[a], W.M. Shannon[a] and P.G. Canonico. U.S. Army Medical Research Institute of Infectious Diseases, Department of Antiviral Studies, Fort Detrick, Frederick, Maryland 21701-5011, U.S.A. and [a]Southern Research Institute, Birmingham, Alabama, U.S.A.

Ribavirin, a broad spectrum antiviral agent, does not inhibit Japanese encephalitis virus (JEV) induced encephalitis or resultant mortality, even though it has exhibited good antiviral activity against JEV in tissue culture (ID_{50} = 42μg/ml). This lack of in vivo efficacy appears to result from the relative inability of ribavirin to cross the blood-brain-barrier. We surmounted this problem by the brain-specific delivery of ribavirin via a redox prodrug, derived from a similar procedure used successfully for a number of other compounds (by N. Bodor, Pharmacol. Ther. (1983) 19,337). The 5' nicotinate ester prodrug of ribavirin was synthesized by a five step route starting from ribavirin. Forty percent of C57BL/6 mice treated intraperitoneally (i.p.) for 9 days with 45 mg/kg/day of prodrug beginning one day prior to infection survived i.p. challenge with 10 LD_{50} of JEV. No mice treated with an identical regimen of placebo or 50 mg/kg/day of unmodified ribavirin survived virus challenge. This prodrug may prove to be the first effective treatment for RNA virus induced encephalitis.

SS1.5

STUDIES ON THE CARRIER-MEDIATED TARGETED DELIVERY OF THE ANTIVIRAL DRUG RIBAVIRIN USING MONOCLONAL ANTIBODIES FOR THE TREATMENT OF PICHINDE VIRUS INFECTIONS. I. CHEMICAL ASPECTS. M. I. Dawson,[1] D. A. O'Krongly,[1] R. W Sidwell,[2] and B. B. Barnett,[2] Life Sciences Division, SRI International, Menlo Park, California, USA (1); and Departments of Animal, Dairy and Veterinary Sciences (2), and of Biology (3), Utah State University, Logan, Utah, USA.

New methods of treatment are required for viral diseases where vaccines are unavailable or currently used drugs are ineffective or must be administered in such high doses that side effects occur. The effectiveness of drugs can be enhanced by delivering them specifically to infected cells. Using this targeting method of delivery would permit lower effective doses of drug, thereby reducing systemic side effects. One method of achieving this goal would be the linkage of antiviral drugs to monoclonal antibodies directed against the antigens expressed on the surface of viruses and on the surface of the virally infected cells.

To establish the validity of the concept of targeted delivery for the treatment of viral infections with antiviral drug-monoclonal antibody conjugates, we are preparing monoclonal antibodies directed against the surface antigens of Pichinde virus strain An4763, conjugating these monoclonal antibodies to ribavirin, and testing the antiviral activity of the conjugates in in vitro and animal model systems.

Supported by USAMRIID Contract No. DAMD17-86-C-6120

SS1.6

STUDIES ON CARRIER-MEDIATED TARGETED DELIVERY OF THE ANTIVIRAL DRUG RIBAVIRIN USING MONOCLONAL ANTIBODIES FOR THE TREATMENT OF PICHINDE VIRUS INFECTIONS. II. BIOLOGICAL DEVELOPMENT. B.B. Barnett[1], R.W. Sidwell[2], M.J. Burns[1], J.H. Huffman[2], and M.I. Dawson[3]. Departments of [1]Biology, and [2]Animal Dairy and Veterinary Sciences, Utah State University, Logan, UT 84322, USA; and [3]Life Sciences Division, SRI International, Menlo Park, CA, 94025, USA.

Many of the basic requirements for evaluating the feasibility of the therapeutic application of monoclonal antibody (MAb)-drug conjugates to viral diseases have been met. First, MAbs specific for viral antigens expressed on the surface of virus infected cells have been produced. Second, antiviral drugs with demonstrated efficacy towards viral biochemical reactions are available. Third, methods for conjugating antiviral drugs to MAbs have been developed. The studies to be described deal with biological methods development utilizing Pichinde virus (an arenavirus), monoclonal antibodies directed towards Pichinde virus glycoproteins expressed on the surface of infected cells and the antiviral drug ribavirin. To be described are development of Pichinde virus in vitro and in vivo models, antiviral assays, the derivation and production of MAbs against Pichinde virus-specified cell surface antigens, and development of detection procedures specific for antibody towards cell surface associated Pichinde virus antigens.

Supported by Contract No. DAMD17-86-C-6120

SS1.7

COMPARISON OF IMMUNOMODULATORS FOR BROAD SPECTRUM ANTIVIRAL ACTIVITY. A.J. Pinto, M. Brinton and P.S. Morahan. Medical College of Pennsylvania and Wistar Institute, Philadelphia, PA, USA.

Several immunomodulators were effective against infection of adult mice with herpes simplex virus type 2 (HSV-2), encephalomyocarditis virus (EMC), Venezualean equine encephalitis alphavirus (VEE), Banzi flavivirus or Caraparu bunyavirus. Similar effectiveness was found among the various immunomodulators, although the viruses did differ in sensitivity. Greatest protection was observed with C. parvum, alpha interferon, and the synthetic immunomodulators MVE-2, CL246,738, ampligen, and avridine in liposomes. Moderate protection was produced with gamma interferon and various microbially derived cell wall materials. Muramyl tripeptide, alone or in liposomes, was ineffective against HSV-2 and EMC. Greatest effects with the immunomodulators were observed with prophylactic treatment, but early or repeated therapeutic treatment with such drugs as CL246,738, ampligen and alpha or gamma interferon was also partially effective. Thus, a variety of chemically diverse agents can produce broad spectrum antiviral protection. Immunomodulator induced alterations in nonspecific immunity may be involved in the protection, but, a common mechanism for the antiviral action has yet to be established. (Supported by ONR N000014-82-K-0669 and DAMD 17-86-C-17).

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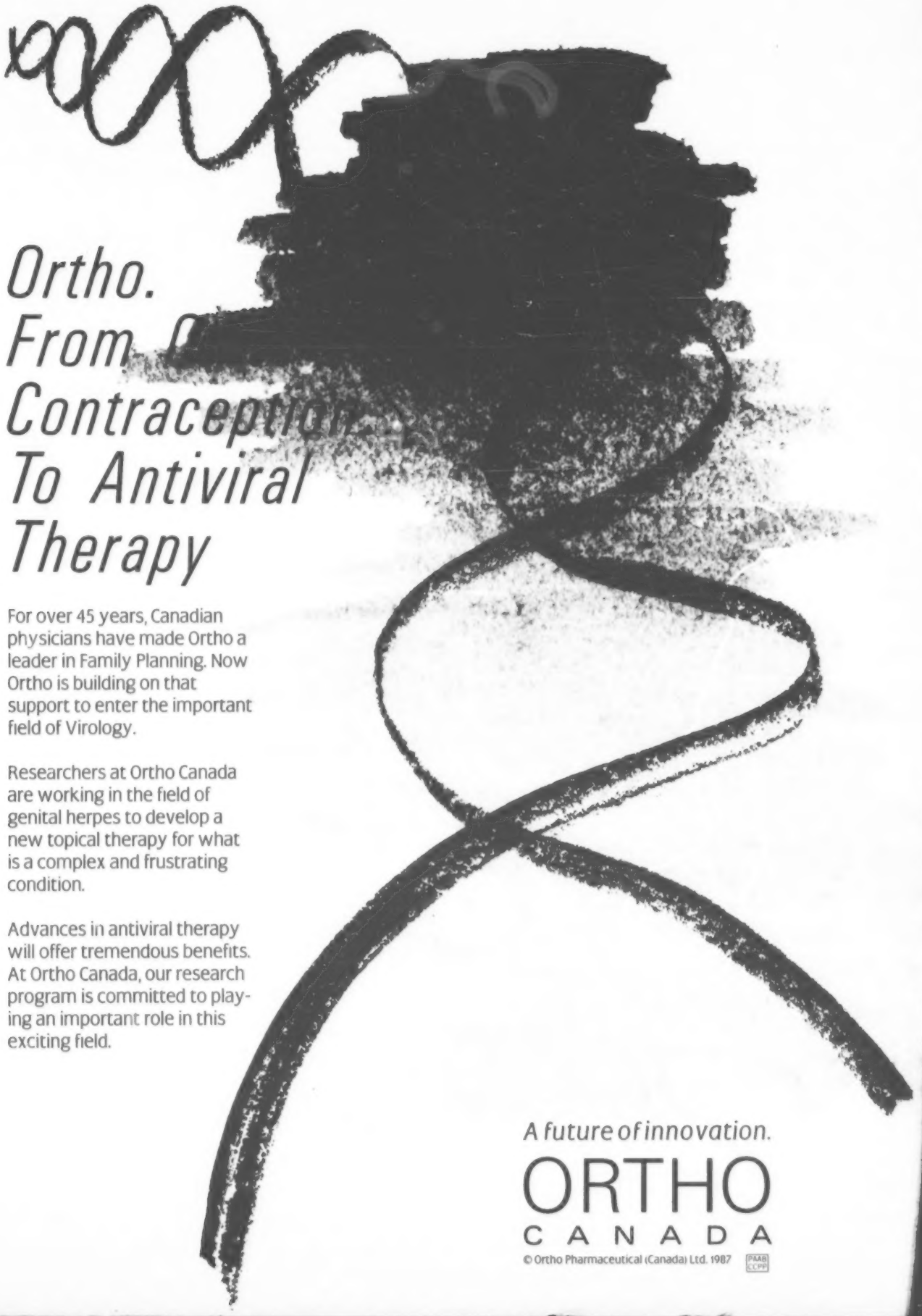

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